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(54) Title: GOODPASTURE ANTIGEN BINDING PROTEIN

(57) Abstract

The present invention provides isolated nucleic acid sequences and expression vectors encoding the Goodpasture antigen binding protein (GPBP), substantially purified GPBP, antibodies against GPBP, and methods for detecting GPBP.

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GOODPASTURE ANTIGEN BINDING PROTEIN

Cross Reference

This application claims priority to U.S. Provisional Patent Application Serial No. 60/121,483, filed February 24, 1999.

Statement of Government Rights

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Field of the Invention

The invention relates to the fields of protein kinases, automimmune disease, apoptosis, and cancer.

Background of the Invention

Goodpasture (GP) disease is an autoimmune disorder described only in humans. In GP patients, autoantibodies against the non-collagenous C-terminal domain (NC1) of the type IV collagen α3 chain ("Goodpasture antigen") cause a rapidly progressive glomerulonephritis and often lung hemorrhage, the two cardinal clinical manifestations of the GP syndrome (see 1 for review. The reference numbers in this section correspond to reference list of Example 1).

The idea that common pathogenic events exist at least for some autoimmune disorders is suggested by the significant number of patients displaying more than one autoimmune disease, and also by the strong and common linkage that some of these diseases show to specific MHC haplotypes (31, 32). The experimental observation that the autoantigen is the leading moiety in autoimmunity and that a limited number of self-components are autoantigenic (31), suggest that these self-components share biological features with important consequences in self/non-self recognition by the immune system.

One possibility is that triggering events, by altering different but specific self-components, would result in abnormal antigen processing. In certain individuals expressing a particular MHC specificity, the abnormal peptides could be recognized by non-tolerized T cells and trigger an immune response (1).

We have previously explored the GP antigen to identify biological features of relevance in autoimmune pathogenesis. Since the NC1 domain is a highly conserved domain among species and between the different type IV collagen α chains (α1-α6) (2), the exclusive involvement of the human α3(IV)NC1 in a natural autoimmune response suggests that this domain has structural and/or biological peculiarities of pathogenic relevance. Consistent with this, the N-terminus of the human antigen is highly divergent, and it contains a unique five-residue motif ($KRGDS^{9}$) that conforms to a functional phosphorylation site for type A protein kinases (3, 4). Furthermore, the human α3 gene, but not the other related human or homologous genes from other species, is alternatively spliced and generates multiple transcripts also containing the phosphorylatable N-terminal region (5-7). Recent studies indicate that the phosphorylation of the N-terminus of the GP antigen by cAMP-dependent protein kinase is up regulated by the presence of the alternative products (see Example 3 below). Specific serine phosphorylation and pre-mRNA alternative splicing are also associated with the biology of other autoantigens including the acetylcholine receptor and myelin basic protein (MBP) (4). The latter is suspected to be the major antigen in multiple sclerosis (MS), another exclusively human autoimmune disease in which the immune system targets the white matter of the central nervous system. GP disease and MS are human disorders that display a strong association with the same HLA class II haplotype (HLA DRB1*1501)(32, 33). This, along with the recent report of death by GP disease of an MS patient carrying this HLA specificity (34), supports the existence of common pathogenic events in these human disorders.

Thus, specific serine/threonine phosphorylation may be a major biological difference between the human GP antigen, the GP antigens of other species, and the homologous domains from the other human α(IV) chains, and might be important in pathogenesis (1, 4).

Therefore, the identification and isolation of the specific serine/threonine kinase that phosphorylates the N-terminal region of the human GP antigen would be very

advantageous for the diagnosis and treatment of GP syndrome, and possibly for other autoimmune disorders.

Summary of the Invention

The present invention fulfills the need in the art for the identification and isolation of a serine/threonine kinase that specifically binds to and phosphorylates the unique N-terminal region of the human GP antigen. In one aspect, the present invention provides nucleic acid sequences encoding various forms of the Goodpasture antigen binding protein (GPBP), as well as recombinant expression vectors operatively linked to the GPBP-encoding sequences.

In another aspect, the present invention provides host cells that have been transfected with the recombinant expression vectors. In a further aspect, the present invention provides substantially purified GPBP and antibodies that selectively bind to GPBP. In still further aspect, the invention provides methods for detecting the presence of GPBP or nucleic acids encoding GPBP.

In a further aspect, the present invention provides methods for detecting the presence of an autoimmune condition or apoptosis, which comprises detecting an increase in the expression of GPBP in a tissue compared to a control tissue.

In another aspect, the present invention provides methods and pharmaceutical compositions for treating an autoimmune disorder, apoptosis, or a tumor, comprising modifying the expression or activity of GPBP in a patient in need thereof.

Brief Description of the Figures

Figure 1. **Nucleotide and derived amino acid sequences of n4'.** The denoted structural features are from 5' to 3'end: the cDNA present in the original clone (HeLa1) (dotted box), which contains the PH homology domain (in black) and the Ser-Xaa-Yaa repeat (in gray); the heptad repeat of the predictable coiled-coil structure (open box) containing the bipartite nuclear localization signal (in gray); and a serine-rich domain (filled gray box). The asterisks denote the positions of in frame stop codons.

Figure 2. **Distribution of GPBP in human tissues (Northern blot) and in eukaryotic species (Southern blot).** A random primed ^{32}P-labeled HeLa1 cDNA probe

was used to identify homologous messages in a Northern blot of poly(A^+)RNA from the indicated human tissues (panel A) or in a Southern blot of genomic DNA from the indicated eukaryotic species (panel B). Northern hybridization was performed under highly stringent conditions to detect perfect matching messages and at low stringency in the Southern to allow the detection of messages with mismatches. No appreciable differences in the quality and amount of each individual poly A+ RNA was observed by denaturing gel electrophoresis or when probing a representative blot from the same lot with human β-actin cDNA. The numbers denote the position and the sizes in kb of the RNA or DNA markers used.

Figure 3. **Experimental determination of the translation start site**. In (A), the two cDNAs present in pc-n4' and pc-FLAG-n4' plasmids used for transient expression are represented as black lines. The relative position of the corresponding predicted (n4') or engineered (FLAG-n4') translation start site is indicated (Met). In (B), the extracts from control (-), pc-n4'(n4') or pc-FLAG-n4' (FLAG-n4') transfected 293 cells were subjected to SDS-PAGE under reducing conditions in 10% gels. The separated proteins were transferred to a PVDF membrane (Millipore) and blotted with the indicated antibodies. The numbers and bars indicate the molecular mass in kDa and the relative positions of the molecular weight markers, respectively.

Figure 4. **Characterization of rGPBP from yeast and 293 cells**. In (A), 1 μg (lane 1) or 100 ng (lanes 2 and 3) of yeast rGPBP were analyzed by reducing SDS-PAGE in a 10% gel. The separated proteins were stained with Coomassie blue (lane 1) or transferred and blotted with anti-FLAG antibodies (lane 2) or Mab14, a monoclonal antibody against GPBP (lane 3). In (B), the cell extracts from GPBP-expressing yeast were analyzed as in A and blotted with anti-FLAG (lane 1), anti-PSer (lane 2), anti-PThr (lane 3) or anti-PTyr (lane 4) monoclonal antibodies respectively. In (C), 200 ng of either yeast rGPBP (lane 1), dephosphorylated yeast rGPBP (lane 2) or 293 cells-derived rGPBP (lane 3) were analyzed as in B with the indicated antibodies. In (D), similar amounts of $H_3{}^{32}PO_4$-labeled non-transfected (lanes 1), stable pc-n4' transfected (lanes 2) or transient pc-FLAG-n4' expressing (lanes 3) 293 cells were lysed, precipitated with the indicated antibodies and analyzed by SDS-PAGE and autoradiography. The molecular weight markers are represented with numbers and bars as in Figure 3. The arrows indicate the position of the rGPBP.

Figure 5. **Recombinant GPBP contains a serine/threonine kinase that specifically phosphorylates the N-terminal region of the human GP antigen.** To assess phosphorylation, approximately 200 ng of yeast rGPBP was incubated with $[\gamma]^{32}$P-ATP in the absence (A and B) or presence of GP antigen-derived material (C). In (A), the mixture was subjected to reducing SDS-PAGE (10% gel) and autoradiographed. In (B), the mixture was subjected to ^{32}P-phosphoamino acid analysis by two-dimensional thin-layer chromatography. The dotted circles indicate the position of ninhydrin stained phosphoamino acids. In (C), the phosphorylation mixtures of the indicated GP-derived material were analyzed by SDS-PAGE (15% gel) and autoradiography (GPpep1 and GPpep1Ala^{9}) or immunoprecipitated with Mab 17, a monoclonal antibody that specifically recognize GP antigen from human and bovine origin, and analyzed by SDS-PAGE (12.5%) and autoradiography (rGP, GP). The relative positions of rGPBP (A), rGP antigen and the native human and bovine GP antigens (C) are indicated by arrows. The numbers and bars refer to molecular weight markers as in previous Figures.

Figure 6. **In-blot renaturation of the serine/threonine kinase present in rGPBP.** Five micrograms of rGPBP from yeast were in-blot renatured. The recombinant material was specifically identified by anti-FLAG antibodies (lane 1) and the *in situ* ^{32}P-incorporation detected by autoradiography (lane 2). The numbers and bars refer to molecular weight markers as in previous Figures. The arrow indicates the position of the 89 kDa rGPBP polypeptide.

Figure 7. **Immunological localization of GPBP in human tissues.** Rabbit serum against the N-terminal region of GPBP (1:50) was used to localize GPBP in human tissues. The tissues shown are kidney (A) glomerulus (B), lung (C), alveolus (D), liver (E), brain (F), testis (G), adrenal gland (H), pancreas (I) and prostate (J). Similar results were obtained using anti-GPBP affinity-purified antibodies or a pool of culture medium from seven different GPBP-specific monoclonal antibodies (anti-GPBP Mabs 3, 4, 5, 6, 8, 10 and 14). Rabbit pre-immune serum did not stain any tissue structure in parallel control studies. Magnification was 40X except in B and D where it was 100X.

Figure 8. **GPBPΔ26 is a splicing variant of GPBP.** (A) Total RNA from normal skeletal muscle was retrotranscribed using primer 53c and subsequently

subjected to PCR with primers 11m-53c (*lane 2*) or 15m-62c (*lane 4*). Control amplifications of a plasmid containing GPBP cDNA using the same pairs of primers are shown in *lanes 1* and *3*. Numbers on the *left* and *right* refer to molecular weight in base pairs. The region missing in the normal muscle transcript was identified and its nucleotide sequence (*lower case*) and deduced amino acid sequence (*upper case*) are shown in (B). A clone of genomic DNA comprising the cDNA region of interest was sequenced and its structure is drawn in (C), showing the location and relative sizes of the 78-bp exon spliced out in GPBPΔ26 (*black box*), adjacent exons (*gray boxes*), and introns (*lines*). The size of both intron and exons is given and the nucleotide sequence of intron-exon boundaries is presented, with consensus for 5' and 3' splice sites shown in *bold case.*

Figure 9. **Differential expression of GPBP and GPBPΔ26.** Fragments representing the 78-bp exon (GPBP) or flanking sequences common to both isoforms (GPBP/GPBPΔ26) were ^{32}P-labeled and used to hybridize human tissue and tumor cell line Northern blots (CLONTECH). The membranes were first hybridized with GPBP-specific probe, stripped and then reanalyzed with GPBP/GPBPΔ26 probe. Washing conditions were less stringent for GPBP-specific probe (0.1% SSPE, 37°C or 55°C) than for the GPBP/GPBPΔ26 (0.1% SSPE, 68°C) to increase GPBP and GPBPΔ26 signals respectively. No detectable signal was obtained for the GPBP probe when the washing program was at 68°C (not shown).

Figure 10. **GPBPΔ26 displays lower phosphorylating activity than GPBP.** (A) Recombinantly-expressed, affinity-purified GPBP (rGPBP) (lanes 1) or rGPBPΔ26 (lanes 2) were subjected to SDS-PAGE under reducing conditions and either Coomasie blue stained (2 μg per lane) or blotted (200ng per lane) with monoclonal antibodies recognizing the FLAG sequence (α-FLAG) or GPBP/GPBPΔ26 (Mab14). (B) 200 ng of rGPBP (lanes 1) or rGPBPΔ26 (lanes 2) were <u>in vitro</u> phosphorylated without substrate to assay auto-phosphorylation (left), or with 5 nmol GPpep1 to measure trans-phosphorylation activity (right). An arrowhead indicates the position of the peptide. (C) 3 μg of rGPBP (lane 1) or rGPBPΔ26 (lane 2) were in-blot renatured as described under Material and Methods. The numbers and bars indicate the molecular mass in kDa and the relative position of the molecular weight markers, respectively.

Figure 11. **rGPBP and rGPBPΔ26 form very active high molecular weight aggregates.** About 300 μg of rGPBP (A) or rGPBPΔ26 (B) were subjected to gel filtration HPLC as described under Material and Methods. *Vertical arrowheads* and *numbers* respectively indicate the elution profile and molecular mass (kDa) of the molecular weight standards used. Larger aggregates eluted in the void volume (I), and the bulk of the material present in the samples eluted in the fractionation range of the column as a second peak between the 669 and 158 kDa markers (II). Fifteen microliters of the indicated minute fractions were subjected to SDS-PAGE and Coomasie blue staining. Five microliters of the same fractions were in vitro phosphorylated as described in Materials and Methods, and the reaction stopped by boiling in SDS sample buffer. The fractions were loaded onto SDS-PAGE, transferred to PVDF and autoradiographed for 1 or 2 hours using Kodak X-Omat films and blotted using anti-FLAG monoclonal antibodies (Sigma).

Figure 12. **Self-interaction of GPBP and GPBPΔ26 assessed by a yeast two-hybrid system.** (A) Cell transfected for the indicated combinations of plasmids were selected on leucine-tryptophan-deficient medium (*-Trp, -Leu*), and independent transformants restreaked onto histidine-deficient plates (*-Trp, -Leu, -His*) in the presence or absence of 1 mM 3-amino-triazole (3-AT), to assess interaction. The picture was taken 3 days after streaking. (B) The bars represent mean values in β-galactosidase arbitrary units of four independent β-galactosidase in-solution assays.

Figure 13. **GPBP is expressed associated with endothelial and glomerular basement membranes.** Paraffin embedded sections of human muscle (A) or renal cortex (B, C) were probed with GPBP-specific antibodies (A,B) or with Mab189, a monoclonal antibody specific for the human α3(IV)NC1 (C). Frozen sections of human kidney (D-F) were probed with Mab17, a monoclonal antibody specific for the α3(IV)NC1 domain (D), GPBP-specific antibodies (E), or sera from a GP patient (F). Control sera (chicken pre-immune and human control) did not display tissue-binding in parallel studies (not shown).

Figure 14. **GPBP is expressed in human but not in bovine and murine renal cortex.** Cortex from human (A, D), bovine (B, E) or murine (C, F) kidney were paraffin

embedded and probed with either GPBP-specific antibodies (A-C) or GPBP/GPBPΔ26-specific antibodies (D-F).

Figure 15. **GPBP is highly expressed in several autoimmune conditions.** Skeletal muscle total RNA from a control individual (lane 1) or from a GP patient (lane 2) was subjected to RT-PCR as in Fig.8, using the oligonucleotides 15m and 62c in the amplification program. Frozen (B-D) or paraffin embedded (E-G) human control skin (B, E) or skin affected by SLE (C, F) or lichen planus (D, G) were probed with GPBP-specific antibodies.

Figure 16. **Phosphorylation of GP alternative splicing products by PKA.** In left panel, equimolecular amounts of rGP (lanes 1), rGPΔV (lanes 2), rGPΔIII (lanes 3) or rGPΔIII/IV/V (lanes 4), equivalent to 500 ng of the GP were phosphorylated at the indicated ATP concentrations. One-fifth of the total phosphorylation reaction mixture was separated by gel electrophoresis and transferred to PVDF, autoradiographed (shown) and the proteins blotted with M3/1, a specific monoclonal antibody recognizing all four species (shown) or using antibodies specific for each individual C-terminal region (not shown). Arrowheads indicate the position of each recombinant protein, from top to bottom, GP, GPΔV and, GPΔIII -GPΔIII/IV/V which displayed the same mobilities. Right panel: purified α3(IV)NC1 domain or hexamer was phosphorylated with PKA and 0.1 μM ATP in the absence (lanes 1) or in the presence of 10 nmol of peptides representing the C-terminal region of either GPΔIII (lanes 2) or GPΔIII/IV/V (lanes 3). Where indicated the phosphorylation mixtures of purified α3(IV)NC1 domain were V8 digested and immunoprecipitated with antibodies specific for the N terminus of the human α3(IV)NC1 domain (3). Bars and numbers indicate the position and sizes (kDa) of the molecular weight markers.

Figure 17. **Sequence alignment of GPΔIII and MBP.** The phosphorylation sites for PKA (boxed) and the structural similarity for the sites at Ser 8 and 9 of MBP and GPΔIII respectively are shown (underlined). The identity (vertical bars) and chemical homology (dots) of the corresponding exon II (bent arrow) of both molecular species are indicated. The complete sequence of GPΔIII from the collagenase cleavage site (72-residues) is aligned with the 69-N terminal residues of MBP comprising the exon I and ten residues of the exon II.

Figure 18. **Phosphorylation of rec mbinant MBP proteins by PKA**. About 200 ng of rMBP (lane 1), or Ser to Ala mutants thereof in position 8 (lane 2) or 57 (lane 3), or rMPBΔII (lane 4) or Ser to Ala mutants thereof in position 8 (lane 5) or 57 (lane 6), were phosphorylated by PKA and 0.1 μM ATP. The mixtures were subjected to SDS-PAGE, transferred to PVDF and autoradiographed (Phosphorylation) and the individual molecular species blotted with monoclonal antibodies against human MBP obtained from Roche Molecular Biochemicals (Western).

Figure 19. **Phosphorylation of recombinant MBP proteins by GPBP**. About 200 ng of rMBP (lane 1), or Ser to Ala mutants thereof in positions 8 (lane 2) or 57 (lane 3), or rMPBΔII (lane 4), or Ser to Ala mutants thereof in positions 8 (lane 5) or 57 (lane 6), were subjected to SDS-PAGE, transferred to PVDF, and the area containing the proteins visualized with Ponceau and stripped out. The immobilized proteins were in situ phosphorylated with rGPBP as described in Materials and Methods, autoradiographed (Phosphorylation) and subsequently blotted as in Fig. 18 (Western).

Figure 20. **Regulation of the GPBP by the C terminal region of GPΔIII**. About 200 ng of rGPBP were in vitro phosphorylated with 150 μM ATP in the absence (lane 1) or in the presence of 5 nmol of GPΔIII-derived peptide synthesized either using Boc- (lane 2) or Fmoc- (lane 3) chemistry. The reaction mixtures were subjected to SDS-PAGE, transferred to PVDF and autoradiographed to asses autophosphorylation, and subsequently blotted with anti-FLAG monoclonal antibodies (Sigma) to determine the amount of recombinant material present (Western).

Detailed Description of the Invention

All references cited are herein incorporated by reference in their entirety.

The abbreviations used herein are: bp, base pair; DTT, dithiothreitol; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid; GP, Goodpasture; rGPΔIII, rGPΔIII/IV/V and rGPΔV, recombinant material representing the alternative forms of the Goodpasture antigen resulting from splicing out exon III, exon III, IV and V or exon V, respectively; GPBP and rGPBP, native and recombinant Goodpasture

antigen binding protein; GPBPΔ26 and rGPBPΔ26, native and recombinant alternative form of the GPBP; GST, glutathione *S*-transferase; HLA, human lymphocyte antigens; HPLC, high performance liquid chromatography; Kb, thousand base pairs; kDa, thousand daltons; MBP, rMBP, native and recombinant 21 kDa myelin basic protein; MBPΔII and rMBPΔII, native and recombinant 18.5 kDa myelin basic protein that results from splicing out exon II; MBPΔV and MBPΔII/V, myelin basic protein alternative forms resulting from splicing out exon V and exons II and V, respectively; MHC, major histocompatibility complex; NC1, non-collagenous domain; PH, pleckstrin homology; PKA, cAMP-dependent protein kinase; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; TBS, tris buffered saline.

Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutshcer, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

As used herein, the term "GPBP" refers to Goodpasture binding protein, and includes both monomers and oligomers thereof. Human (SEQ ID NO:2), mouse (SEQ ID NO:4), and bovine GPBP sequences (SEQ ID NO:6) are provided herein.

As used herein, the term "GPBPΔ26" refers to Goodpasture binding protein deleted for the 26 amino acid sequence shown in SEQ ID NO:14, and includes both monomers and oligomers thereof. Human (SEQ ID NO:8), mouse (SEQ ID NO:10), and bovine GPBP sequences (SEQ ID NO:12) are provided herein.

As used herein the term "GPBPpep1" refers to the 26 amino acid peptide shown in SEQ ID NO:14, and includes both monomers and oligomers thereof.

As used herein, the term "GP antigen" refers to the α3 NC1 domain of type IV collagen.

As used herein, "MBP" refers to myelin basic protein.

In one aspect, the present invention provides isolated nucleic acids that encode GPBP, GPBPΔ26, and GPBPpep1, and mutants or fragments thereof. In one embodiment, the isolated nucleic acids comprise sequences substantially similar to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25, or fragments thereof.

In another aspect, the present invention provides isolated nucleic acids that encode alternative products of the GP antigen or MBP. In one embodiment, the isolated nucleic acids comprise sequences that encode peptides substantially similar to SEQ ID NO:43 and SEQ ID NO:44.

The phrase "substantially similar " is used herein in reference to the nucleotide sequence of DNA or RNA, or the amino acid sequence of protein, having one or more conservative or non-conservative variations from the disclosed sequences, including but not limited to deletions, additions, or substitutions, wherein the resulting nucleic acid and/or amino acid sequence is functionally equivalent to the sequences disclosed herein. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same protein disclosed herein. For example, functionally equivalent DNAs encode proteins that are the same as those disclosed herein or that have one or more conservative amino acid variations, such as substitution of a non-polar residue for another non-polar residue or a charged residue for a similarly charged residue. These changes include those recognized by those of skill in the art as substitutions that do not substantially alter the tertiary structure of the protein.

In practice, the term substantially similar means that DNA encoding two proteins hybridize to one another under conditions of moderate to high stringency, and encode proteins that have either the same sequence of amino acids, or have changes in sequence that do not alter their structure or function. As used herein, substantially similar sequences of nucleotides or amino acids share at least about 70% identity, more preferably at least about 80% identity, and most preferably at least about 90% identity. It is recognized, however, that proteins (and DNA or mRNA encoding such proteins)

containing less than the above-described level of homology arising as splice variants or that are modified by conservative amino acid substitutions (or substitution of degenerate codons) are contemplated to be within the scope of the present invention.

Stringency of hybridization is used herein to refer to conditions under which nucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_M) of the hybrids. T_M decreases approximately 1-1.5°C with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions. Thus, as used herein, moderate stringency refers to conditions that permit hybridization of those nucleic acid sequences that form stable hybrids in 0.1% SSPE at 37°C or 55°C, while high stringency refers to conditions that permit hybridization of those nucleic acid sequences that form stable hybrids in 0.1%SSPE at 65°C. It is understood that these conditions may be duplicated using a variety of buffers and temperatures and that they are not necessarily precise. Denhardt's solution and SSPE (see, e.g., Sambrook, Fritsch, and Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art, as are other suitable hybridization buffers.

The isolated nucleic acid sequence may comprise an RNA, a cDNA, or a genomic clone with one or more introns. The isolated sequence may further comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals.

In another aspect, the present invention provides recombinant expression vectors comprising nucleic acid sequences that express GPBP, GPBPΔ26, or GPBPpep1, and mutants or fragments thereof. In one embodiment, the vectors comprise nucleic acid sequences that are substantially similar to the sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25, or fragments thereof.

In another aspect, the present invention provides recombinant expression vectors comprising nucleic acid sequences that express peptides that are substantially similar to the amino acid sequence shown in SEQ ID NO:43, SEQ ID NO:44, or peptide fragments thereof.

"Recombinant expression vector" includes vectors that operatively link a nucleic acid coding region or gene to any promoter capable of effecting expression of the gene product. The promoter sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive). The construction of expression vectors for use in transfecting prokaryotic cells is also well known in the art, and thus can be accomplished via standard techniques. (See, for example, Sambrook, Fritsch, and Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989; *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX)

The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In a preferred embodiment, the expression vector comprises a plasmid. However, the invention is intended to include other expression vectors that serve equivalent functions, such as viral vectors.

In a further aspect, the present invention provides host cells that have been transfected with the recombinant expression vectors disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably transfected. Such transfection of expression vectors into prokaryotic and eukaryotic cells can be accomplished via any technique known in the art, including but not limited to standard bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated-, or viral mediated transfection. (See, for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press; *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY),

In a still further aspect, the present invention provides substantially purified GPBP, GPBPΔ26, and GPBPpep1, and mutants or fragments thereof. In one embodiment, the amino acid sequence of the substantially purified protein is substantially similar to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or peptide fragments thereof.

In another aspect, the present invention provides substantially purified alternative products of the GP antigen and MBP. In one embodiment, the amino acid sequence of the substantially purified polypeptide is substantially similar to SEQ ID NO:43, SEQ ID NO:44, or peptide fragments thereof.

As used herein, the term "substantially purified" means that the protein has been separated from its in vivo cellular environments. Thus, the protein can either be purified from natural sources, or recombinant protein can be purified from the transfected host cells disclosed above. In a preferred embodiment, the proteins are produced by the transfected cells disclosed above, and purified using standard techniques. (See for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press.)) The protein can thus be purified from prokaryotic or eukaryotic sources. In various further preferred embodiments, the protein is purified from bacterial, yeast, or mammalian cells.

The protein may comprise additional sequences useful for promoting purification of the protein, such as epitope tags and transport signals. Examples of such epitope tags include, but are not limited to FLAG (Sigma Chemical, St. Louis, MO), myc (9E10) (Invitrogen, Carlsbad, CA), 6-His (Invitrogen; Novagen, Madison, WI), and HA (Boehringer Manheim Biochemicals). Examples of such transport signals include, but are not limited to, export signals, secretory signals, nuclear localization signals, and plasma membrane localization signals.

In another aspect, the present invention provides antibodies that selectively bind to GPBP, GPBPΔ26, or GPBPpep1. In one aspect, the antibodies selectively bind to a protein comprising a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or peptide fragments thereof. Such antibodies can be produced by immunization of a host

animal with either the complete GPBP, or with antigenic peptides thereof. The antibodies can be either polyclonal or monoclonal.

In another aspect, the present invention provides antibodies that selectively bind to a polypeptide comprising an amino acid sequence substantially similar to a sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO:50, SEQ ID NO:54, or antigenic fragments thereof. The antibodies can be either polyclonal or monoclonal.

Antibodies can be made by well-known methods, such as described in Harlow and Lane, Antibodies; A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1988). In one example, preimmune serum is collected prior to the first immunization. Substantially purified proteins of the invention, or antigenic fragments thereof, together with an appropriate adjuvant, is injected into an animal in an amount and at intervals sufficient to elicit an immune response. Animals are bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. At about 7 days after each booster immunization, or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20° C. Polyclonal antibodies against the proteins and peptides of the invention can then be purified directly by passing serum collected from the animal through a column to which non-antigen-related proteins prepared from the same expression system without GPBP-related proteins bound.

Monoclonal antibodies can be produced by obtaining spleen cells from the animal. (See Kohler and Milstein, Nature 256, 495-497 (1975)). In one example, monoclonal antibodies (mAb) of interest are prepared by immunizing inbred mice with the proteins or peptides of the invention, or an antigenic fragment thereof. The mice are immunized by the IP or SC route in an amount and at intervals sufficient to elicit an immune response. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of by the intravenous (IV) route. Lymphocytes, from antibody positive mice are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner under conditions which will allow the formation of

stable hybridomas. The antibody producing cells and fusion partner cells are fused in polyethylene glycol at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells and are screened for antibody production by an immunoassay such as solid phase immunoradioassay. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, Soft Agar Techniques, in Tissue Culture Methods and Applications, Kruse and Paterson, Eds., Academic Press, 1973.

To generate such an antibody response, the proteins of the present invention are typically formulated with a pharmaceutically acceptable carrier for parenteral administration. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing Corynebacterium parvum and tRNA. The formulation of such compositions, including the concentration of the polypeptide and the selection of the vehicle and other components, is within the skill of the art.

The term antibody as used herein is intended to include antibody fragments thereof which are selectively reactive with the proteins and peptides of the invention, or fragments thereof. Antibodies can be fragmented using conventional techniques, and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

In a further aspect, the invention provides methods for detecting the presence of the proteins or peptides of the invention in a protein sample, comprising providing a protein sample to be screened, contacting the protein sample to be screened with an antibody against the proteins or peptides of the invention, and detecting the formation of antibody-antigen complexes. The antibody can be either polyclonal or monoclonal as described above, although monoclonal antibodies are preferred. As used herein, the term "protein sample" refers to any sample that may contain the proteins or peptides of the invention, and fragments thereof, including but not limited to tissues and portions thereof, tissue sections, intact cells, cell extracts, purified or partially purified protein

samples, bodily fluids, nucleic acid expression libraries. Accordingly, this aspect of the present invention may be used to test for the presence of GPBP, GPBPΔ26, GPBPpep1, or alternative products of the GP antigen in these various protein samples by standard techniques including, but not limited to, immunolocalization, immunofluorescence analysis, Western blot analysis, ELISAs, and nucleic acid expression library screening, (See for example, Sambrook et al, 1989.) In one embodiment, the techniques may determine only the presence or absence of the protein or peptide of interest. Alternatively, the techniques may be quantitative, and provide information about the relative amount of the protein or peptide of interest in the sample. For quantitative purposes, ELISAs are preferred.

Detection of immunocomplex formation between the proteins or peptides of the invention, or fragments thereof, and their antibodies or fragments thereof, can be accomplished by standard detection techniques. For example, detection of immunocomplexes can be accomplished by using labeled antibodies or secondary antibodies. Such methods, including the choice of label are known to those ordinarily skilled in the art. (Harlow and Lane, Supra). Alternatively, the polyclonal or monoclonal antibodies can be coupled to a detectable substance. The term "coupled" is used to mean that the detectable substance is physically linked to the antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase. Examples of suitable prosthetic-group complexes include streptavidin/biotin and avidin/biotin. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. An example of a luminescent material includes luminol. Examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Such methods of detection are useful for a variety of purposes, including but not limited to detecting an autoimmune condition, identifying cells targeted for or undergoing apoptosis, immunolocalization of the proteins of interest in a tissue sample, Western blot analysis, and screening of expression libraries to find related proteins.

In yet another aspect, the invention provides methods for detecting the presence in a sample of nucleic acid sequences encoding the GPBP, GPBPΔ26, GPBPpep1, or alternative products of the GP antigen comprising providing a nucleic acid sample to be screened, contacting the sample with a nucleic acid probe derived from the isolated nucleic acid sequences of the invention, or fragments thereof, and detecting complex formation.

As used herein, the term "sample" refers to any sample that may contain GPBP-related nucleic acid, including but not limited to tissues and portions thereof, tissue sections, intact cells, cell extracts, purified or partially purified nucleic acid samples, DNA libraries, and bodily fluids. Accordingly, this aspect of the present invention may be used to test for the presence of GPBP mRNA or DNA in these various samples by standard techniques including, but not limited to, in situ hybridization, Northern blotting, Southern blotting, DNA library screening, polymerase chain reaction (PCR) or reverse transcription-PCR (RT-PCR). (See for example, Sambrook et al, 1989.) In one embodiment, the techniques may determine only the presence or absence of the nucleic acid of interest. Alternatively, the techniques may be quantitative, and provide information about the relative amount of the nucleic acid of interest in the sample. For quantitative purposes, quantitative PCR and RT-PCR are preferred. Thus, in one example, RNA is isolated from a sample, and contacted with an oligonucleotide derived from the nucleic acid sequence of interest, together with reverse transcriptase under suitable buffer and temperature conditions to produce cDNAs from the GPBP-related RNA. The cDNA is then subjected to PCR using primer pairs derived from the nucleic acid sequence of interest. In a preferred embodiment, the primers are designed to detect the presence of the RNA expression product of SEQ ID NO:5, and the amount of GPBP gene expression in the sample is compared to the level in a control sample.

For detecting the nucleic acid sequence of interest, standard labeling techniques can be used to label the probe, the nucleic acid of interest, or the complex between the probe and the nucleic acid of interest, including, but not limited to radio-, enzyme-, chemiluminescent-, or avidin or biotin-labeling techniques, all of which are well known in the art. (See, for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San

Diego, CA); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA)).

Such methods of nucleic acid detection are useful for a variety of purposes, including but not limited to diagnosing an autoimmune condition, identifying cells targeted for or undergoing apoptosis, in situ hybridization, Northern and Southern blot analysis, and DNA library screening.

As demonstrated in the following examples, GPBP shows preferential expression in tissue structures that are commonly targeted in naturally-occurring automimmune responses, and is highly expressed in several autoimmune conditions, including but not limited to Goodpasture Syndrome (GP), systemic lupus erythematosus (SLE), and lichen planus. Furthermore, following a similar experimental approach to that described below, recombinant proteins representing autoantigens in GP disease (α3 Type IV collagen), SLE (P1 ribosomal phosphoprotein and Sm-D1 small nuclear ribonucleoproteins) and dermatomyositis (hystididyl-tRNA synthetase) were shown to be in vitro substrates of GPBP.

Thus, in a preferred embodiment, detection of GPBP expression is used to detect an autoimmune condition. A sample that is being tested is compared to a control sample for the expression of GPBP, wherein an increased level of GPBP expression indicates the presence of an autoimmune condition. In this embodiment, it is preferable to use antibodies that selectively bind to GPBPpep1, which is present in GPBP but not in GPBPΔ26.

Furthermore, as shown in the accompanying examples, GPBP is down-regulated in tumor cell lines, and the data suggest that GPBP/GPBPΔ26 are likely to be involved in cell signaling pathways that induce apoptosis, which may be up-regulated during autoimmune pathogenesis and down-regulated during cell transformation to prevent autoimmune attack to transformed cells during tumor growth. Thus, the detection methods disclosed herein can be used to detect cells that are targeted for, or are undergoing apoptosis.

In another aspect, the present invention provides a method for treating an autoimmune disorder, a tumor, or for preventing cell apoptosis comprising modification of the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1 in a patient in need thereof. Modifying the

expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1 can be accomplished by using specific inducers or inhibitors of GPBP expression or activity, GPBP antibodies, gene or protein therapy using GP or myelin basic protein alternative products, cell therapy using host cells expressing GP or myelin basic protein alternative products, antisense therapy, or other techniques known in the art. In a preferred embodiment, the method further comprises administering a substantially purified alternative product of the GP antigen or MBP to modify the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1. As used herein, "modification of expression or activity" refers to modifying expression or activity of either the RNA or protein product.

In a further aspect, the present invention provides pharmaceutical compositions, comprising an amount effective of substantially purified alternative products of the GP antigen or MBP to modify the expression or activity of GPBP RNA or protein, and a pharmaceutically acceptable carrier.

For administration, the active agent is ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be mixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidine, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

The present invention may be better understood with reference to the accompanying examples that are intended for purposes of illustration only and should not be construed to limit the scope of the invention, as defined by the claims appended hereto.

Example 1: Characterizati n of GPBP

Here we report the cloning and characterization of a novel type of serine/threonine kinase that specifically binds to and phosphorylates the unique N-terminal region of the human GP antigen.

MATERIALS AND METHODS

Synthetic polymers-*Peptides.* GPpep1, KGKRGDSGSPATWTTRGFVFT (SEQ ID NO:26), representing residues 3-23 of the human GP antigen and GPpep1Ala^9, KGKRGDAGSPATWTTRGFVFT (SEQ ID NO:27), a mutant Ser^9 to Ala^9 thereof, were synthesized by MedProbe and CHIRON. FLAG peptide, was from Sigma.

Oligonucleotides. The following as well as several other GPBP-specific oligonucleotides were synthesized by Genosys and GIBCO BRL:

ON-GPBP-54m:
TCGAATTCACCATGGCCCCACTAGCCGACTACAAGGACGACGATG
ACAAG (SEQ ID NO: 28).

ON-GPBP-55c:
CCGAGCCCGACGAGTTCCAGCTCTGATTATCCGACATCTTGTCATCG
TCG (SEQ ID NO:29).

ON-HNC-B-N-14m: CGGGATCCGCTAGCTAAGCCAGGCAAGGATGG (SEQ ID NO:30).

ON-HNC-B-N-16c: CGGGATCCATGCATAAATAGCAGTTCTGCTGT (SEQ ID NO:31).

Isolation and characterization of cDNA clones encoding human GPBP- Several human λ-gt11 cDNA expression libraries (cye, fetal and adult lung, kidney and HeLa S3, from CLONTECH) were probed for cDNAs encoding proteins interacting with GPpep1. Nitrocellulose filters (Millipore) prepared following standard immunoscreening procedures were blocked and incubated with 1-10 nmoles per ml of GPpep1 at 37°C. Specifically bound GPpep1 was detected using M3/1A monoclonal antibodies (7). A single clone was identified in the HeLa-derived library (HeLa1). Specificity of fusion protein binding was confirmed by similar binding to recombinant

eukaryotic human GP antigen. The EcoRI cDNA insert of HeLa1 (0.5-kb) was used to further screen the same library and to isolate overlapping cDNAs. The largest cDNA (2.4-kb) containing the entire cDNA of HeLa1 (n4') was fully sequenced.

Northern and Southern blots-Pre-made Northern and Southern blots (CLONTECH) were probed with HeLa1 cDNA following manufacturer instructions.

Plasmid construction, expression and purification of recombinant proteins-*GPBP-derived material.* The original λ-gt11 HeLa1 clone was expressed as a lysogen in E. Coli Y1089 (8). The corresponding β-galactosidase-derived fusion protein containing the N-terminal 150 residues of GPBP was purified from the cell lysate using an APTG-agarose column (Boehringer). The EcoRI 2.4-kb fragment of n4' was subcloned in Bluescribe M13+ vector (Stratagene) (BS-n4'), amplified and used for subsequent cloning. A DNA fragment containing (from 5' to 3'), an EcoRI restriction site, a standard Kozak consensus for translation initiation, a region coding for a tag peptide sequence (FLAG, DYKDDDDK (SEQ ID NO:32)), and the sequence coding for the first eleven residues of GPBP including the predicted Met_i and a Ban II restriction site, was obtained by hybridizing ON-GPBP-54m and ON-GPBP-55c, and extending with modified T_7 DNA polymerase (Amersham). The resulting DNA product was digested with EcoRI and BanII, and ligated with the BanII/EcoRI cDNA fragment of BS-n4' in the EcoRI site of pHIL-D2 (Invitrogen) to produce pHIL-FLAG-n4'. This plasmid was used to obtain Mut^s transformants of the GS115 strain of *Pichia pastoris* and to express FLAG-tagged recombinant GPBP (rGPBP) either by conventional liquid culture or by fermentation procedures (*Pichia* Expression Kit, Invitrogen). The cell lysates were loaded onto an anti-FLAG M2 column (Sigma), the unbound material washed out with Tris buffered saline (TBS, 50 mM Tris-HCl, pH 7.4, 150 mM NaCl) or salt-supplemented TBS (up to 2M NaCl), and the recombinant material eluted with FLAG peptide. For expression in cultured human kidney-derived 293 cells (ATCC 1573-CRL), the 2.4- or 2.0-kb EcoRI cDNA insert of either BS-n4' or pHIL-FLAG-n4' was subcloned in pcDNA3 (Invitrogen) to produce pc-n4' and pc-FLAG-n4' respectively. When used for transient expression, 18 hours after transfection the cells were lysed with 3.5-4 $\mu l/cm^2$ of chilled lysis buffer (1% Nonidet P-40 or Triton-X100, 5mM EDTA and 1 mM PMSF in TBS) with or without 0.1% SDS, depending on whether the lysate was to be used for SDS-PAGE or FLAG-purification, respectively.

For FLAG purification, the lysate of four to six 175 cm^2 culture dishes was diluted up to 50 ml with lysis buffer and purified as above. For stable expression, the cells were similarly transfected with pc-n4' and selected for three weeks with 800 μg/ml of G418. For bacterial recombinant expression, the 2.0-kb EcoRI cDNA fragment of pHIL-FLAG-n4' was cloned in-frame downstream of the glutathione *S*-transferase (GST)-encoding cDNA of pGEX-5x-1 (Pharmacia). The resulting construct was used to express GST-GPBP fusion protein in DH5α cells (9).

GP antigen-derived material. Human recombinant GP antigen (rGP) was produced in 293 cells using the pRc/CMV-BM40 expression vector containing the α3-specific cDNA between ON-HNC-B-N-14m and ON-HNC-B-N-16c. The expression vector is a pRc/CMV (Invitrogen)-derived vector provided by Billy G. Hudson (Kansas University Medical Center) that contains cDNA encoding an initiation Met, a BM40 signal peptide followed by a tag peptide sequence (FLAG), and a polylinker cloning site. To obtain α3-specific cDNA, a polymerase chain reaction was performed using the oligonucleotides above and a plasmid containing the previously reported α3(IV) cDNA sequence (3) as template (clone C2). For stable expression of rGP, 293 cells were transfected with the resulting construct (fα3VLC) and selected with 400 μg/ml of G418. The harvested rGP was purified using an anti-FLAG M2 column.

All the constructs were verified by restriction mapping and nucleotide sequencing.

Cell culture and DNA transfection-Human 293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Transfections were performed using the calcium phosphate precipitation method of the Profection Mammalian Transfection Systems (Promega). Stably transfected cells were selected by their resistance to G418. Foci of surviving cells were isolated, cloned and amplified.

Antibody production-*Polyclonal antibodies against the N-terminal region of GPBP.* Cells expressing HeLa1 λ-gt11 as a lysogen were lysed by sonication in the presence of Laemmli sample buffer and subjected to electrophoresis in a 7.5% acrylamide preparative gel. The gel was stained with Coomassie blue and the band containing the fusion protein of interest excised and used for rabbit immunization (10). The anti-serum was tested for reactivity using APTG-affinity purified antigen. To

obtain affinity-purified antibodies, the anti-serum was diluted 1:5 with TBS and loaded onto a Sepharose 4B column containing covalently bound affinity purified antigen. The bound material was eluted and, unless otherwise indicated, used in the immunochemical studies.

Monoclonal antibodies against GPBP. Monoclonal antibodies were produced essentially as previously reported (7) using GST-GPBP. The supernatants of individual clones were analyzed for antibodies against rGPBP.

***In vitro* phosphorylation assays**-About 200 ng of rGPBP were incubated overnight at 30°C in 25 mM β-glycerolphosphate (pH 7.0), 0.5 mM EDTA, 0.5 mM EGTA, 8 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM DTT and 0.132 μM γ-^{32}P-ATP, in the presence or absence of 0.5-1 μg of protein substrates or 10 nmoles of synthetic peptides, in a total volume of 50 μl.

***In vivo* phosphorylation assays**-Individual wells of a 24-well dish were seeded with normal or with stably pc-n4' transfected 293 cells. When the cells were grown to the desired density, a number of wells of the normal 293 cells were transfected with pc-FLAG-n4'. After 12 hours, the culture medium was removed, 20 μCi/well of $H_3{}^{32}PO4$ in 100 μl of phosphate-free DMEM added, and incubation continued for 4 hours. The cells were lysed with 300 μl/well of TBS containing 1% Triton X-100, 2 mM EDTA, 1 mM PMSF, 50 mM NaF and 0.2 mM vanadate, and extracted with specific antibodies and Protein A-Sepharose. When anti-GPBP serum was used, the lysate was pre-cleared using pre-immune serum and Protein A-Sepharose.

***In vitro* dephosphorylation of rGPBP**-About 1 μg of rGPBP was dephosphorylated in 100 μl of 10 mM Tris-acetate (pH 7.5), 10 mM magnesium acetate and 50 mM potassium acetate with 0.85 U of calf intestine alkaline phosphatase (Pharmacia) for 30 min at 30°C.

Renaturation assays-In-blot renaturation assays were performed using 1-5 μg of rGPBP as previously described (11).

Nucleotide sequence analysis- cDNA sequence analyses were performed by the dideoxy chain termination method using [α]^{35}S-dATP, modified T_7 DNA polymerase (Amersham) and universal or GPBP-specific primers (8-10).

^{32}P-Phosphoamino acid analysis-Immunopurified rGPBP or HPLC gel-filtration fractions thereof containing the material of interest were phosphorylated, hydrolyzed and analyzed in one dimensional (4) or two dimensional thin layer chromatography (12). When performing two dimensional analysis, the buffer for the first dimension was formic acid:acetic acid:water (1:3.1:35.9) (pH 1.9) and the buffer for the second dimension was acetic acid:pyridine:water (2:0.2:37.8) (pH 3.5). Amino acids were revealed with ninhydrin, and ^{32}P-phosphoamino acids by autoradiography.

Physical methods and immunochemical techniques-SDS-PAGE and Western-blotting were performed as in (4). Immunohistochemistry studies were done on human multi-tissue control slides (Biomeda, Biogenex) using the ABC peroxidase method (13).

Computer analysis-Homology searches were carried out against the GenBank and SwissProt databases with the BLAST 2.0 (14) at the NCBI server, and against the TIGR Human Gene Index database for expressed sequence tags, using the Institute for Genomic Research server. The search for functional patterns and profiles was performed against the PROSITE database using the ProfileScan program at the Swiss Institute of Bioinformatics (15). Prediction of coiled-coil structures was done at the Swiss Institute for Experimental Cancer Research using the program Coils (16) with both 21 and 28 residue windows.

RESULTS

Molecular cloning of GPBP-To search for proteins specifically interacting with the divergent N-terminal region of the human GP antigen, a 21-residue peptide (GPpep1; SEQ ID NO:26)), encompassing this region and flanking sequences, and specific monoclonal antibodies against it were combined to screen several human cDNA expression libraries. More than 5 x 10^6 phages were screened to identify a single HeLa-derived recombinant encoding a fusion protein specifically interacting with GPpep1 without disturbing antibody binding.

Using the cDNA insert of the original clone (HeLa1), we isolated a 2.4-kb cDNA (n4') that contains 408-bp of 5'-untranslated sequence, an open reading frame (ORF) of

1872-bp encoding 624 residues, and 109-bp of 3'-untranslated sequence (Fig. 1) (SEQ ID NO:1-2). Other structural features are of interest. First, the predicted polypeptide (hereinafter referred to as GPBP) has a large number of phosphorylatable (17.9%) and acidic (16%) residues unequally distributed along the sequence. Serine, which is the most abundant residue (9.3%), shows preference for two short regions of the protein, where it comprises nearly 40% of the amino acids, compared to an average of less than 7% throughout the rest of the polypeptide chain. It is also noteworthy that the more N-terminal, serine-rich region consists mainly of a Ser-Xaa-Yaa repeat. Acidic residues are preferentially located at the N-terminal three-quarters of the polypeptide, with nearly 18% of the residues being acidic. These residues represent only 9% in the most C-terminal quarter of the polypeptide, resulting in a polypeptide chain with two electrically opposite domains. At the N-terminus, the polypeptide contains a pleckstrin homology (PH) domain, which has been implicated in the recruitment of many signaling proteins to the cell membrane where they exert their biological activities (17). Finally, a bipartite nuclear targeting sequence (18) exists as an integral part of a heptad repeat region that meets all the structural requirements to form a coiled-coil (16).

Protein data bank searches revealed homologies almost exclusively within the approximately 100 residues at the N-terminal region harboring the PH domain. The PH domain of the oxysterol-binding protein is the most similar, with an overall identity of 33.5% and a similarity of 65.2% with GPBP. In addition, the *Caenorhabditis elegans* cosmid F25H2 (accession number Q93569) contains a hypothetical ORF that displays an overall identity of 26.5% and a similarity of 61% throughout the entire protein sequence, indicating that similar proteins are present in lower invertebrates. Several human expressed sequence tags (accession numbers AA287878, AA287561, AA307431, AA331618, AA040134, AA158618, AA040087, AA122226, AA158617, AA121104, AA412432, AA412433, AA282679 and N27578) possess a high degree of nucleotide identity (above 98%) with the corresponding stretches of the GPBP cDNA, suggesting that they represent human GPBP. Interestingly, the AA287878 EST shows a gap of 67 nucleotides within the sequence corresponding to the GPBP 5'-untranslated region, suggesting that the GPBP pre-mRNA is alternatively spliced in human tissues (not shown).

The distribution and expression of the GPBP gene in human tissues was first assessed by Northern blot analysis (Fig. 2, panel A). The gene is expressed as two major mRNAs species between 4.4-kb and 7.5-kb in length and other minor species of shorter lengths. The structural relationship between these multiple mRNA species is not known and their relative expression varies between tissues. The highest expression level is seen in striated muscle (skeletal and heart), while lung and liver show the lowest expression levels.

Southern blot studies analysis of genomic DNA from different species indicated that homologous genes exist throughout phylogeny (Fig. 2, panel B). Consistent with the human origin of the probe, the hybridization intensities decreased in a progressive fashion as the origin of the genomic DNA moves away from humans in evolution.

Experimental determination of the translation start site-To experimentally confirm the predicted ORF, eukaryotic expression vectors containing either the 2.4-kb of cDNA of n4', or only the predicted ORF tagged with a FLAG sequence (Fig. 3A), were used for transient expression assays in 293 cells. The corresponding extracts were analyzed by immunoblot using GPBP- or FLAG-specific antibodies. The GPBP-specific antibodies bind to a similar major polypeptide in both transfected cells, but only the polypeptide produced by the engineered construct expressed the FLAG sequence (Fig. 3B). This located the translation start site of the n4' cDNA at the predicted Met and confirmed the proposed primary structure. Furthermore, the recombinant polypeptides displayed a molecular mass higher than expected (80 versus 71 kDa) suggesting that GPBP undergoes post-translational modifications.

Expression and characterization of yeast rGPBP-Yeast expression and FLAG-based affinity-purification were combined to produce rGPBP (Fig. 4A). A major polypeptide of ~89 kDa, along with multiple related products displaying lower M_r, were obtained. The recombinant material was recognized by both anti-FLAG and GPBP-specific antibodies, guaranteeing the fidelity of the expression system. Again, however, the M_r displayed by the major product was notably higher than predicted and even higher than the M_r of the 293 cell-derived recombinant material, supporting the idea that GPBP undergoes important and differential post-translational modifications. Since phosphorylatable residues are abundant in the polypeptide chain, we investigated the existence of phosphoamino acids in the recombinant materials. By using monoclonal or

polyclonal (not shown) antibodies against phosphoserine (Pser), phosphothreonine (PThr) and phosphotyrosine (PTyr), we identified the presence of all three phosphoresidues either in yeast rGPBP (Fig. 4B) or in 293 cell-derived material (not shown). The specificity of the antibodies was further assessed by partially inhibiting their binding by the addition of 5-10 mM of the corresponding phosphoamino acid (not shown). This suggests that the phosphoresidue content varies depending upon the cell expression system, and that the M_r differences are mainly due to phosphorylation. Dephosphorylated yeast-derived material consistently displayed similar M_r to the material derived from 293 cells, and phosphoamino acid content correlates with SDS-PAGE mobilities (Fig.4C). As an *in vivo* measurement, the phosphorylation of rGPBP in the 293 cells was assessed (Fig. 4D). Control cells (lanes 1) and cells expressing rGPBP in a stable (lanes 2) or transient (lanes 3) mode were cultured in the presence of $H_3{}^{32}PO_4$. Immunoprecipitated recombinant material contained ^{32}P, indicating that phosphorylation of GPBP occurred *in vivo* and therefore is likely to be a physiological process.

The rGPBP is a serine/threonine kinase that phosphorylates the N-terminal region of the human GP antigen-Although GPBP does not contain the conserved structural regions required to define the classic catalytic domain for a protein kinase, the recent identification and characterization of novel non-conventional protein kinases (19-27) encouraged the investigation of its phosphorylating activity. Addition of [γ^{32}P]ATP to rGPBP (either from yeast or 293 cells (not shown)) in the presence of Mn^{2+} and Mg^{2+} resulted in the incorporation of ^{32}P as PSer and PThr in the major and related products recognized by both anti-FLAG and specific antibodies (Fig. 5A and B), indicating that the affinity-purified material contains a Ser/Thr protein kinase. To further characterize this activity, GPpep1, GPpep1Ala^9 (a GPpep1 mutant with Ser^9 replaced by Ala), native and recombinant human GP antigens, and native bovine GP antigen were assayed (Fig. 5C). Affinity-purified rGPBP phosphorylates all human-derived material to a different extent. However, in similar conditions, no appreciable ^{32}P-incorporation was observed in the bovine-derived substrate. The lower ^{32}P incorporation displayed by GPpep1Ala^9 when compared with GPpep1, and the lack of phosphorylation of the bovine antigen, indicates that the kinase present in rGPBP discriminates between human and bovine antigens, and that Ser^9 is a target for the kinase.

Although the purification system provides high quality material, the presence of contaminants with a protein kinase activity could not be ruled out. The existence of contaminants was also suggested by the presence of a FLAG-containing 40 kDa polypeptide, which displayed no reactivity with specific antibodies nor incorporation of ^{32}P in the phosphorylation assays (Fig. 4A and 5A). To precisely identify the polypeptide harboring the protein kinase activity, we performed *in vitro* kinase renaturation assays after SDS-PAGE and Western-blotted (Fig. 6). We successfully combined the use of specific antibodies (lane 1) and autoradiographic detection of *in situ* ^{32}P-incorporation (lane 2), and identified the 89 kDa rGPBP material as the primary polypeptide harboring the Ser/Thr kinase activity. The lack of ^{32}P-incorporation in the rGPBP-derived products, as well as in the 40 kDa contaminant, further supports the specificity of the renaturation assays and locates the kinase activity to the 89 kDa polypeptide. Recently, it has been shown that traces of protein kinases intimately associated with a polypeptide can be released from the blot membrane, bind to, and phosphorylate the polypeptide during the labeling step (28). To assess this possibility in our system, we performed renaturation studies using a small piece of membrane containing the 89 kDa polypeptide, either alone or together with membrane pieces representing the different regions of the blot lane. We observed similar ^{32}P-incorporation at the 89 kDa polypeptide regardless of the co-incubated pieces (not shown), indicating that if there are co-purified protein kinases in our sample they are not phosphorylating the 89 kDa polypeptide in the renaturation assays unless they co-migrate. Co-migration does not appear to be a concern, however, since rGPBP deletion mutants (GPBPΔ26 and R3; see below) displaying different mobilities also have kinase activities and could be similarly in-blot renatured (not shown).

Immunohistochemical localization of the novel kinase-To investigate GPBP expression in human tissues we performed immunohistochemical studies using specific polyclonal (Fig.7) or monoclonal antibodies (not shown). Although GPBP is widely expressed in human tissues, it shows tissue and cell-specificity. In kidney, the major expression is found at the tubule epithelial cells and the glomerular mesangial cells and podocytes. At the lung alveolus, the antibodies display a linear pattern suggestive of a basement membrane localization, along with staining of pneumocytes. Liver shows low expression in the parenchyma, but high expression in biliary ducts. Expression in the central nervous system is observed in the white matter, but not in the neurons of the brain.

In testis, a high expression in the spermatogonium contrasts with the lack of expression in Sertoli cells. The adrenal gland shows a higher level of expression in cortical cells versus the medullar. In the pancreas, GPBP is preferentially expressed in Langerhans islets versus the exocrine moiety. In prostate, GPBP is expressed in the epithelial cells but not in the stroma (Fig. 7). Other locations with high expression of GPBP are striated muscle, epithelial cells of intestinal tract, and Purkinje cells of the cerebellum (not shown). In general, in tissues where GPBP is highly expressed the staining pattern is mainly diffuse cytosolic. However in certain locations there is, in addition, an important staining reinforcement at the nucleus (spermatogonium), at the plasma membrane (pneumocyte, hepatocyte, prostate epithelial cells, white matter) or at the extracellular matrix (alveolus) (Fig. 7).

DISCUSSION

Our data show that GPBP is a novel, non-conventional serine/threonine kinase. We also present evidence that GPBP discriminates between human and bovine GP antigens, and targets the phosphorylatable region of human GP antigen *in vitro*. Several lines of evidence indicate that the 89 kDa polypeptide is the only kinase in the affinity purified rGPBP. First, we found no differences in auto- or trans-phosphorylation among rGPBP samples purified in the presence of 150 mM, 0.5 M, 1 M or 2 M salt (not shown), suggesting that rGPBP does not carry intimately bound kinases. Second, there is no FLAG-containing, yeast-derived kinase in our samples, since material purified using GPBP-specific antibodies shows no differences in phosphorylation (not shown). Third, a deletion mutant (GPBPΔ26; see below) displays reduced auto- and trans-phosphorylation activities (not shown), demonstrating that the 89 kD polypeptide is the only portion of the rGPBP with the ability to carry out phosphate transfer.

Although GPBP is not homologous to other non-conventional kinases, they share some structural features including an N-terminal α-helix coiled-coil (26, 27), serine-rich motifs (24), high phosphoamino acids content (27), bipartite nuclear localization signal (27), and the absence of a typical nucleotide or ATP binding motif (24, 27).

Immunohistochemistry studies show that GPBP is a cytosolic polypeptide also found in the nucleus, associated with the plasma membrane and likely at the extracellular matrix associated with the basement membrane, indicating that it contains the structural

requirements to reach all these destinations. The nuclear localization signal and the PH domain confer to it the potential to reach the nucleus and the cell membrane, respectively (17, 29, 30). Although GPBP does not contain the structural requirements to be exported, the 5'-end untranslated region of its mRNA includes an upstream ORF of 130 residues with an in-frame stop codon at the beginning (Fig. 1). A mRNA editing process inserting a single base pair (U) would generate an operative in-frame start site and an ORF of 754-residues containing an export signal immediately downstream of the edited Met (not shown). Polyclonal antibodies against a synthetic peptide representing part of this hypothetical extra-sequence (PRSARCQARRRRGGRTSS (SEQ ID NO:33)) display a linear vascular reactivity in human tissues suggestive of an extracellular basement membrane localization (data not shown).

Alternatively, a splicing phenomenon could generate transcripts with additional unidentified exon(s) that would provide the structural requirements for exportation. The multiple cellular localization, the high content in PTyr, and the lack of tyrosine kinase activity *in vitro*, suggest that GPBP is itself the target of specific tyrosine kinase(s) and therefore likely involved in specific signaling cascade(s).

As discussed above, specific serine phosphorylation, as well as pre-mRNA alternative splicing, are associated with the biology of several autoantigens, including the GP antigen, acetylcholine receptor and myelin basic protein (MBP) (4). The latter is suspected to be the major antigen in multiple sclerosis (MS), another exclusively human autoimmune disease in which the immune system targets the white matter of the central nervous system. GP disease and MS are human disorders that display a strong association with the same HLA class II haplotype (HLA DRB1*1501)(32, 33). This, along with the recent report of death by GP disease of a MS patient carrying this HLA specificity (34), supports the existence of common pathogenic events in these human disorders.

Phosphorylation of specific serines has been shown to change intracellular proteolysis (35-40). Conceivably, alterations in protein phosphorylation can affect processing and peptide presentation, and thus mediate autoimmunity. GP antigen-derived peptide presentation by the HLA-DR15 depends more on processing than on preferences of relatively indiscriminate DR15 molecules (41), suggesting that if processing is influenced by abnormal phosphorylation, the resulting peptides would likely be presented by this HLA. Our more recent data indicate that in both the GP and MBP systems, the

production of alternative splicing products serves to regulate the phosphorylation of specific and structurally homologous PKA sites, suggesting that this or a closely related kinase is the *in vivo* phosphorylating enzyme. Alterations in the degree of antigen phosphorylation, caused either by an imbalance in alternative products, or by the action of an intruding kinase that deregulates phosphorylation of the same motifs, could lead to an autoimmune response in predisposed individuals. rGPBP phosphorylates the human GP antigen at a major PKA phosphorylation site in an apparently unregulated fashion, since the presence of specific alternative products of the GP antigen did not affect phosphorylation of the primary antigen by GPBP (not shown).

Although GPBP is ubiquitously expressed, in certain organs and tissues it shows a preference for cells and tissue structures that are target of common autoimmune responses: the Langerhans cells (type I diabetes); the white matter of the central nervous system (multiple sclerosis); the biliary ducts (primary biliary cirrhosis); the cortical cells of the adrenal gland (Addison disease); striated muscle cells (myasthenia gravis); spermatogonium (male infertility); Purkinje cells of the cerebellum (paraneoplasic cerebellar degeneration syndrome); and intestinal epithelial cells (pernicious anemia, autoimmune gastritis and enteritis). All the above observations point to this novel kinase as an attractive candidate to be considered when envisioning a model for human autoimmune disease.

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Example 2: GPBP Alternative Splicing

Here we report the existence of two isoforms of GPBP that are generated by alternative splicing of a 78-base pair (bp) long exon that encodes a 26-residue serine-rich motif. Both isoforms, GPBP and GPBPΔ26, exist as high molecular aggregates that result from polypeptide self-aggregation. The presence of the 26-residue peptide in the polypeptide chain results in a molecular species that self-interacts more efficiently and forms aggregates with higher specific activity. Finally, we present evidences supporting the observation that GPBP is implicated in human autoimmune pathogenesis.

MATERIAL AND METHODS.

Synthetic polymers:

Peptides. GPpep1, KGKRGDSGSPATWTTRGFVFT (SEQ ID NO:26), is described in Example 1. GPBPpep1, PYSRSSSMSSIDLVSASDDVHRFSSQ (SEQ ID NO:14), representing residues 371-396 of GPBP was synthesized by Genosys.

Oligonucleotides. The following oligonucleotides were synthesized by Life Technologies, Inc., 5' to 3': ON-GPBP-11m, G CGG GAC TCA GCG GCC GGA TTT TCT (SEQ ID NO:34); ON-GPBP-15m, AC AGC TGG CAG AAG AGA C (SEQ ID NO:35); ON-GPBP-20c, C ATG GGT AGC TTT TAA AG (SEQ ID NO; 36); ON-GPBP-22m, TA GAA GAA CAG TCA CAG AGT GAA AAG G (SEQ ID NO;37); ON-GPBP-53c, GAATTC GAA CAA AAT AGG CTT TC (SEQ ID NO:38); ON-GPBP-56m, CCC TAT AGT CGC TCT TC (SEQ ID NO:39); ON-GPBP-57c, CTG GGA GCT GAA TCT GT (SEQ ID NO:40); ON-GPBP-62c, GTG GTT CTG CAC CAT CTC TTC AAC (SEQ ID NO:41); ON-GPBP-Δ26, CA CAT AGA TTT GTC CAA AAG GTT GAA GAG ATG GTG CAG AAC (SEQ ID NO:42).

Reverse transcriptase and polymerase chain rection (RT-PCR). Total RNA was prepared from different control and GP tissues as described in (15). Five micrograms of total RNA was retrotranscribed using Ready-To-Go You-Prime First-Strand beads (Amersham Pharmacia Biotech) and 40 pmol of ON-GPBP-53c. The corresponding cDNA was subjected to PCR using the pairs of primers ON-GPBP-11m/ON-GPBP-53c or ON-GPBP-15m/ON-GPBP-62c. The identity of the products obtained with 15m-62c

was further confirmed by Alu I restriction. To specifically amplify GPBP transcripts, PCR was performed using primers ON-GPBP-15m/ON-GPBP-57c.

Northern hybridization studies. Pre-made human multiple-tissue and tumor cell-line Northern Blots (CLONTECH) were probed with a cDNA containing the 78-bp exon present only in GPBP or with a cDNA representing both isoforms. The corresponding cDNAs were obtained by PCR using the pair of primers ON-GPBP-56m and ON-GPBP-57c using GPBP as a template, or with primers ON-GPBP-22m and ON-GPBP-20c, using GPBPΔ26 as a template. The resulting products were random-labeled and hybridized following the manufacturers' instructions.

Plasmid construction, expression and purification of recombinant proteins. The plasmid pHIL-FLAG-n4', used for recombinant expression of FLAG-tagged GPBP in *Pichia pastoris* has been described elsewhere (4). The sequence coding for the 78-bp exon was deleted by site-directed mutagenesis using ON-GPBP-Δ26 to generate the plasmid pHIL-FLAG-n4'Δ26. Expression and affinity-purification of recombinant GPBP and GPBPΔ26 was done as in (4).

Gel-filtration HPLC. Samples of 250 µl were injected into a gel filtration PE-TSK-G4000SW HPLC column equilibrated with 50 mM Tris-HCl pH 7.5, 150 mM NaCl. The material was eluted from the column at 0.5 ml/min, monitored at 220 nm and minute fractions collected.

In vitro phosphorylation assays. The auto-, trans-phosphorylation and in-blot renaturation studies were performed as in Example 1.

Antibodies and immunochemical techniques. Polyclonal antibodies were raised by in chicken against a synthetic peptide (GPBPpep1) representing the sequence coded by the 78-bp exon (Genosys). Egg yolks were diluted 1:10 in water, the pH adjusted to 5.0. After 6 hours at 4°C, the solution was clarified by centrifugation (25 min at 10000 x *g* at 4°C) and the antibodies precipitated by adding 20 % (w/v) of sodium sulfate at 20.000 x g, 20'. The pellets were dissolved in PBS (1 ml per yolk) and used for immunohistochemical studies. The production of antibodies against GPBP/GPBPΔ26 or against α3(IV)NC1 domain are discussed above (see also 4, 13).

Sedimentation velocity. Determination of sedimentation velocities were performed in an Optima XL-A analytical ultracentrifuge (Beckman Instruments Inc.), equipped with a VIS-UV scanner, using a Ti60 rotor and double sector cells of Epon-charcoal of 12

mm optical path-length. Samples of ca. 400 µl were centrifuged at 30,000 rpm at 20°C and radial scans at 220 nm were taken every 5 min. The sedimentation coefficients were obtained from the rate of movement of the solute boundary using the program XLAVEL (supplied by Beckman).

Sedimentation equilibrium. Sedimentation equilibrium experiments were done as described above for velocity experiments with samples of 70 µl, and centrifuged at 8,000 rpm. The experimental concentration gradients at equilibrium were analyzed using the program EQASSOC (Beckman) to determine the corresponding weight average molecular mass. A partial specific volumes of 0.711 cm^3/g for GPBP and 0.729 cm^3/g for GPBPΔ26 were calculated from the corresponding amino acid compositions.

Physical methods and immunochemical techniques. SDS-PAGE and Western blotting were performed under reducing conditions as previously described (3). Immunohistochemistry studies were done on formalin fixed paraffin embedded tissues using the ABC peroxidase method (4) or on frozen human biopsies fixed with cold acetone using standard procedures for indirect immunofluorescence.

Two hybrid studies. Self-interaction studies were carried out in *Saccharomyces cerevisiae* (HF7c) using pGBT9 and pGAD424 (CLONTECH) to generate GAL4 binding and activation domain-fusion proteins, respectively. Interaction was assessed following the manufacture's recommendations. β-galactosidase activity was assayed with X-GAL (0.75 mg/ml) for in situ and with ortho-nitrophenyl β-D galactopyranoside (0.64 mg/ml) for the in-solution determinations.

RESULTS

Identification of two spliced GPBP variants. To characterize the GPBP species in normal human tissues, we coupled reverse transcription to a polymerase chain reaction (RT-PCR) on total RNA from different tissues, using specific oligonucleotides that flank the full open reading frame of GPBP. A single cDNA fragment displaying lower size than expected was obtained from skeletal muscle-derived RNA (Fig.8A), and from kidney, lung, skin, or adrenal gland-derived RNA (not shown). By combining nested PCR re-amplifications and endonuclease restriction mapping, we determined that all the RT-PCR products corresponded to the same molecular species (not shown). We fully sequenced the 2.2-Kb of cDNA from human

muscle and found it identical to HeLa-derived material except for the absence of 78-nucleotides (positions 1519-1596), which encode a 26-residues motif (amino acids 371-396) (Fig. 8B). We therefore named this more common isoform of GPBP as GPBPΔ26.

To investigate whether the 78-bp represent an exon skipped transcript during pre-mRNA processing, we used this cDNA fragment to probe a human-derived genomic library and we isolated a ~14-Kb clone. By combining Southern blot hybridization and PCR, the genomic clone was characterized and a contiguous DNA fragment of 12482-bp was fully sequenced (SEQ ID 25). The sequence contained (from 5' to 3'), 767-bp of intron sequence, a 93-bp exon, an 818-bp intron, the 78-bp exon sequence of interest, a 9650-bp intron, a 96-bp exon and a 980-bp intron sequence (Fig. 8C). The exon-intron boundaries determined by comparing the corresponding DNA and cDNA sequences meet the canonical consensus for 5' and 3' splice sites (Fig 8C) (5), thus confirming the exon nature of the 78-bp sequence. The GPBP gene was localized to chromosome 5q13 by fluorescence in situ hybridization (FISH) using the genomic clone as a probe (not shown).

The relative expression of GPBP in human-derived specimens was assessed by Northern blot analysis, using either the 78-bp exon or a 260-bp cDNA representing the flanking sequence of 78-bp (103-bp 5' and 157-bp 3') present in both GPBP and GPBPΔ26 (Fig. 9). The 78-bp containing the molecular species of interest were preferably expressed in striated muscle (both skeletal and heart) and brain, and poorly expressed in placenta, lung and liver. In contrast to GPBPΔ26, the GPBP was expressed at very low levels in kidney, pancreas and cancer cell lines.

All the above indicates that GPBP is expressed at low levels in normal human tissues, and that the initial lack of detection by RT-PCR of GPBP can be attributed to a preferential amplification of the more abundant GPBPΔ26. Indeed, the cDNA of GPBP could be amplified from human tissues (skeletal muscle, lung, kidney, skin and adrenal gland) when the specific RT-PCR amplifications were done using 78-bp exon-specific oligonucleotides (not shown). This also suggests that GPBPΔ26 mRNA is the major transcript detected in Northern blot studies when using the cDNA probe representing both GPBP and GPBPΔ26.

Recombinant expression and functional characterization of GPBPΔ26. To investigate whether the absence of the 26-residue serine-rich motif would affect the biochemical properties of GPBP, we expressed and purified both isoforms (rGPBP and rGPBPΔ26), and assessed their auto- and trans-phosphorylation activities (Fig. 10). As reported above for rGPBP (see also 4), rGPBPΔ26 is purified as a single major polypeptide and several related minor products (Fig.10 A). However, the number and relative amounts of the derived products vary compared to rGPBP, and they display M_r on SDS-PAGE that cannot be attributed simply to the 26-residue deletion. This suggests that the 26-residue motif has important structural and functional consequences that could account for the reduced in-solution auto- and trans-phosphorylation activities displayed by rGPBPΔ26 (Fig.10B). Interestingly, the differences in specific activity shown in the in-solution assays were not evident when autophosphorylation was assessed in-blot after SDS-PAGE and renaturation, suggesting that the 26-residue motif likely has important functional consequences at the quaternary structure level. Renaturation studies further showed that phosphate transfer activities reside in the major polypeptides representing the proposed open reading frames, and are not detectable in derived minor products.

rGPBP and rGPBP-26 exist as very active high molecular weight aggregates. Gel filtration analysis of affinity-purified rGPBP or rGPBPΔ26 yielded two chromatographic peaks (I and II), both displaying higher MW than expected for the individual molecular species, as determined by SDS-PAGE studies (89 kDa and 84 kDa, respectively) (Fig. 11). The bulk of the recombinant material eluted as a single peak between the 158 kDa and the 669 kDa molecular weight markers (peak II), while limited amounts of rGPBP and only traces of rGPBPΔ26 eluted in peak I (>1000 kDa). Aliquots of fractions representing each chromatographic profile were subjected to SDS-PAGE and stained, or incubated in the presence of $^{32}P[\gamma]$ ATP, and analyzed by immunoblot and autoradiography. Along with the major primary polypeptide, every chromatographic peak contained multiple derived products of higher or lower sizes indicating that the primary polypeptide associates to form high molecular weight aggregates that are stabilized by covalent and non-covalent bonds (not shown). The kinase activity also exhibited two peaks coinciding with the chromatographic profiles.

However, peak I showed a much higher specific activity than peak II, indicating that these high molecular weight aggregates contained a much more active form of the kinase. Equal volumes of rGPBP fractions number 13 and 20 exhibited comparable phosphorylating activity, even though the protein content is approximately 20 times lower in fraction 13, as estimated by Western blot and Coomasie blue staining (Fig. 11A). The specific activities of rGPBP and rGPBPΔ26 at peak II are also different, and are consistent with the studies shown for the whole material, thus supporting the hypothesis that the presence of the 26-rediue serine-rich motif renders a more active kinase. These results also suggest that both rGPBP and rGPBPΔ26 exist as oligomers under native conditions, and that both high molecular weight aggregate formation and specific activity are greatly dependent on the presence of the 26-residue serine-rich motif. Analytical centrifugation analysis of rGPBP revealed that peak I contained large aggregates (over 10^7 Da). Peak II of rGPBP contained a homogenous population of 220 ± 10 kDa aggregates, likely representing trimers with a sedimentation coefficient of 11S. Peak II of rGPBPΔ26 however consisted of a more heterogenous population that likely contains several oligomeric species. The main population (ca. 80%) displayed a weight average molecular mass of 310 ± 10 kDa and a coefficient of sedimentation of 14S.

GPBP and GPBPΔ26 self-interact in a yeast two-hybrid system. To assess the physiological relevance of the self-aggregation, and to determine the role of the 26-residue motif, we performed comparative studies using a two-hybrid interaction system in yeast. In this type of study, the polypeptides whose interaction is under study are expressed as a part of a fusion protein containing either the activation or the binding domains of the transcriptional factor GAL4. An effective interaction between the two fusion proteins through the polypeptide under study would result in the reconstitution of the transcriptional activator and the subsequent expression of the two reporter genes, Lac Z and His3, allowing colony color detection and growth in a His-defective medium, respectively. We estimated the intensity of interactions by the growth-rate in histidine-defective medium, in the presence of different concentrations of a competitive inhibitor of the His3 gene product (3-AT), and a quantitative colorimetric liquid β-galactosidase assay. A representative experiment is presented in Fig. 12. When

assaying GPBPΔ26 for self-interaction, a significant induction of the reporter genes was observed, while no expression was detectable when each fusion protein was expressed alone or with control fusion proteins. The insertion of the 26-residue motif in the polypeptide to obtain GPBP resulted in a notable increase in polypeptide interaction. All of the above data indicate that GPBPΔ26 self-associates *in vivo*, and that thc insertion of the 26-residues into the polypeptide chain yields a more interactive molecular species.

GPBP is highly expressed in human but not in bovine and murine glomerulus and alveolus. We have shown that GPBP/GPBPΔ26 is preferentially expressed in human cells and tissues that are commonly targeted in naturally occurring autoimmune responses. To specifically investigate the expression of GPBP, we raised polyclonal antibodies against a synthetic peptide representing the 26-residue motif characteristic of this kinase isoform, and used it for immunohistochemical studies on frozen or formalin fixed paraffin embedded human tissues (Fig 13). In general, these antibodies showed more specificity than the antibodies recognizing both isoforms for the tissue structures that are target of autoimmune responses such as the biliary ducts, the Langerhans islets or the white matter of the central nervous system (not shown). Nevertheless, the most remarkable finding was the presence of linear deposits of GPBP-selective antibodies around the small vessels in every tissue studied (A), suggesting that GPBP is associated with endothelial basement membranes. Consequently, at the glomerulus, the anti-GPBP antibodies displayed a vascular pattern closely resembling the glomerular basement membrane staining yielded either by monoclonal antibodies specifically recognizing the α3(IV)NC1 (compare 13B with 13C and 13D), or by circulating GP autoantibodies (compare 13E and 13F). These observations further supported the initial observation that GPBP is expressed in tissue structures targeted in natural autoimmune responses, suggesting that the expression of GPBP is a risk factor and makes the host tissue vulnerable to an autoimmune attack.

To further assess this hypothesis, we investigated the presence of GPBP and GPBPΔ26 in the glomerulus of two mammals that naturally do not undergo GP disease compared to human (Fig.14). GPBP-specific antibodies failed to stain the glomerulus of both bovine or murine specimens (compare 14A with 14B and 14C) while antibodies

recognizing the N-terminal sequence common to both GPBP and GPBPΔ26 stained these structures in all three species, although with different distributions and intensities (14D-14F). In bovine renal cortex, GPBPΔ26 was expressed at a lower rate than in human, but showed similar tissue distribution. In murine samples, however, GPBPΔ26 displayed a tissue distribution closely resembling that of GPBP in human glomerulus. Similar results were obtained when studying the alveolus in the three different species (not shown). To rule out that the differences in antibody detection was due to primary structure differences rather than to a differential expression, we determined the corresponding primary structures in these two species by cDNA sequencing. Bovine and mouse GPBP (SEQ ID NOS:3-6 and 9-12) displayed an overall identity with human material of 97.9% and 96.6% respectively. Furthermore, the mouse 26-residue motif was identical to human while bovine diverged only in one residue. Finally, and similarly to human, we successfully amplified GPBP cDNA from mouse or bovine kidney total RNA using oligonucleotides specific for the corresponding 78-bp exons, indicating that GPBP is expressed at very low levels not detectable by immunochemical techniques.

GPBP is highly expressed in several autoimmune conditions. We analyzed several tissues from different GP patients by specific RT-PCR to assess GPBP/GPBPΔ26 mRNA levels. As in control kidneys, the major expressed isoform in GP kidneys was GPBPΔ26. However, in the muscle of one of the patients, GPBP was preferentially expressed, whereas GPBPΔ26 was the only isoform detected in control muscle samples (Fig. 15 A). Since we did not have kidney samples from this particular patient, we could not assess GPBP/GPBPΔ26 expression in the corresponding target organ. For similar reasons, we could not assess GPBP/GPBPΔ26 levels in the muscle of the patients in which kidneys were studied. Muscle cells express high levels of GPBP/GPBPΔ26 (see Northern blot in Fig. 9), and they comprise the bulk of the tissue. In contrast, the expression of GPBP/GPBPΔ26 in the kidney was much less, and the glomerulus was virtually the only kidney structure expressing the GPBP isoform (see Fig. 13). The glomerulus is a relatively less abundant structure in kidney than the myocyte is in muscle, and the glomerulus is the structure targeted by immune attack in GP pathogenesis. These factors, together with the preferential amplification of the more

abundant and shorter messages when performing RT-PCR studies, could account for the lack of detection of GPBP in both normal and GP kidneys, thus precluding the assessment of GPBP expression at the glomerulus during pathogenesis. Nevertheless, the increased levels of GPBP in a GP patient suggest that GPBP/GPBPΔ26 expression is altered during GP pathogenesis, and that augmented GPBP expression has a pathogenic significance in GP disease.

To investigate the expression of GPBP and GPBPΔ26 in autoimnune pathogenesis, we studied cutaneous autoimmune processes and compared them with control samples representing normal skin or non-autoimmune dermatitis (Fig. 15). Control samples displayed a limited expression of GPBP in the most peripheral keratinocytes (15B, 15E), while keratinocytes expanding from stratum basale to corneum expressed abundant GPBP in skin affected by systemic lupus erythematosus (SLE) (15C, 15F) or lichen planus (15D, 15G). GPBP was preferentially expressed in cell surface structures that closely resembled the blebs previously described in cultured keratinocytes upon UV irradiation and apoptosis induction (6). In contrast, antibodies recognizing both GPBP and GPBPΔ26 yielded a diffuse cytosolic pattern through the whole epidermis in both autoimmune affected or control samples (not shown). These data indicate that in both control and autoimmune-affected keratinocytes, GPBPΔ26 was expressed at the cytosol and that the expression did not significantly vary during cell differentiation. In contrast, mature keratinocytes were virtually the only GPBP expressing cells. However, bleb formation and expression of GPBP was observed in the early stages of differentiation in epidermis affected by autoimmune responses (15C, 15D, 15F, 15G). This further supports previous observations indicating that aberrant apoptosis at the basal keratinocytes is involved in the pathogenesis of autoimmune processes affecting skin (7), and suggests that apoptosis and GPBP expression are linked in this human cell system.

DISCUSSION

Alternative pre-mRNA splicing is a fundamental mechanism for differential gene expression that has been reported to regulate the tissue distribution, intracellular localization, and function of different protein kinases (8-11). In this regard, and closely

resembling GPBP, B-Raf exists as multiple spliced variants, in which the presence of specific exons renders more interactive, efficient and oncogenic kinases (12).

Although it is evident that rGPBPΔ26 still bears the uncharacterized catalytic domain of this novel kinase, both auto- and trans-phosphorylating activities are greatly reduced when compared to rGPBP. Gel filtration and two hybrid experiments provide some insights into the mechanisms that underlie such a reduced phosphate transfer activity. About 1-2% of rGPBP is organized in very high molecular weight aggregates that display about one third of the phosphorylating activity of rGPBP, indicating that high molecular aggregation renders more efficient quaternary structures. Recombinant GPBPΔ26, with virtually no peak I material, consistently displayed a reduced kinase activity. However, aggregation does not seem to be the only mechanism by which the 26-residues increases specific activity, since the rGPBPΔ26 material present in peak II also shows a reduced phosphorylating activity when compared to homologous fractions of rGPBP. One possibility is that rGPBP-derived aggregates display higher specific activities because of quaternary structure strengthening caused by the insertion of the 26-residue motif. The oligomers are kept together mainly by very strong non-covalent bonds, since the bulk of the material appears as a single polypeptide in non-reducing SDS-PAGE, and the presence of either 8 M urea or 6 M guanidine had little effect on chromatographic gel filtration profiles (not shown). How the 26-residue motif renders a more strengthened and active structure remains to be clarified. Conformational changes induced by the presence of an exon encoded motif that alter the activation status of the kinase have been proposed for the linker domain of the Src protein (24) and exons 8b and 10 of B-Raf (12). Alternatively, the 26-residue motif may provide the structural requirements such as residues whose phosphorylation may be necessary for full activation of GPBP.

We have reported (13) that the primary structure of the GP antigen (α3(IV)NC1) is the target of a complex folding process yielding multiple conformers. Isolated conformers are non-minimum energy structures specifically activated by phosphorylation for supramolecular aggregation and likely quaternary structure formation. In GP patients, the α3(IV)NC1 shows conformational alterations and a reduced ability to mediate the disulfide stabilization of the collagen IV network. The GP antibodies, in turn, demonstrate

stronger affinity towards the patient α3(IV)NC1 conformers, indicating that conformationally altered material caused the autoimmune response. Therefore, it seems that in GP disease an early alteration in the conforming process of the α3(IV)NC1 could generate altered conformers for which the immune system is not tolerant, thus mediating the autoimmune response.

Other evidence (Raya et al., unpublished results) indicates that phosphorylation is the signal that drives the folding of the α3(IV)NC1 into non-minimum energy ends. In this scenario, three features of the human α3(IV)NC1 system are of special pathogenic relevance when compared to the corresponding antigen systems from species that, like bovine or murine, do not undergo spontaneous GP disease. First, the N-terminus of the human α3(IV)NC1 contains a motif that is phosphorylatable by PKA and also by GPBP (see above, and also 2-4). Second, the human gene generates multiples alternative products by alternative exon splicing (14,15). Exon skipping generates alternative products with divergent C-terminal ends that up-regulate the in vitro PKA phosphorylation of the primary α3(IV)NC1 product (See below Example 3). Third, the human GPBP is expressed associated with glomerular and alveolar basement membranes, the two main targets in GP disease. The phosphorylation-dependent conforming process is also a feature of non-pathogenic NC1 domains (13), suggesting that the phosphorylatable N-terminus, the alternative splicing diversification, and the expression of GPBP at the glomerular and alveolar basement membranes, are all exclusively human features that place the conformation process of α3(IV)NC1 in a vulnerable condition. The four independent GP kidneys studied expressed higher levels of GP antigen alternative products (15; Bernal and Saus, unpublished results), and an augmented expression of GPBP were found in a GP patient (see above). Both increased levels of alternative GP antigen products and GPBP are expected to have consequences in the phosphorylation-dependent conformational process of the α3(IV)NC1, and therefore with pathogenic potential.

GPBP is highly expressed in skin targeted by natural autoimmune responses. In the epidermis, GPBP is associated with cell surface blebs characteristic of the apoptosis-mediated differentiation process that keratinocytes undergo during maturation from basale to corneum strata (22, 23). Keratinocytes from SLE patients

show a remarkably heightened sensitivity to UV-induced apoptosis (6, 18, 20), and augmented and premature apoptosis of keratinocytes has been reported to exist in SLE and dermatomyositis (7). Consistently, we found apoptotic bodies expanding from basal to peripheral strata of the epidermis in several skin autoimmune conditions including discoid lupus (not shown), SLE and lichen planus. Autoantigens, and modified versions thereof are clustered in the cell surface blebs of apoptotic keratinocytes (6,18,20). Apoptotic surface blebs present autoantigens (21), and likely release modified versions to the circulation (16-20). It has been suggested that the release of modified autoantigens from apoptotic bodies could be the immunizing event that mediates systemic autoimmune responses mediating SLE and scleroderma (18,19).

Our evidence indicates that both GPBP and GPBPΔ26 are able to act in vitro as protein kinases, with GPBP being a more active isoform than GPBPΔ26. Furthermore, recombinant material representing GPBP or GPBPΔ26 purified from yeast or from human 293 cells contained an associated proteolytic activity that specifically degrades the α3(IV)NC1 domain (unpublished results). The proteolytic activity operates on α3(IV)NC1 produced in an eukaryotic expression system, but not on recombinant material produced in bacteria (unpublished results), indicating that α3(IV)NC1 processing has some conformational or post-translational requirements not present in prokaryotic recombinant material. Finally, it has been reported that several autoantigens undergo phosphorylation and degradation in apoptotic keratinocytes (20). While not being limited to an exact mechanism, we propose, in light of all of the above data, that the machinery assembling GPBP at the apoptotic blebs likely performs a complex modification of the autoantigens that includes phosphorylation, conformational changes and degradation. Accordingly, recombinant protein representing autoantigens in SLE (P1 ribosomal phosphoprotein and Sm-D1 small nuclear ribonucleoproteins) and in dermatomyositis (hystidil-tRNA synthetase) were in vitro substrates of GPBP (unpublished results).

The down-regulation in cancer cell lines of GPBP, suggest that the cell machinery harboring GPBP/GPBPΔ26 is likely involved in signaling pathways inducing programmed cell death. The corresponding apoptotic pathway could be up regulated during autoimmune pathogenesis to cause an altered antigen presentation in

individuals carrying specific MHC haplotypes; and down regulated during cell transformation to prevent autoimmune attack to the transformed cells during tumor growth.

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Example 3. Regulation of Human Autoantigen Phosphorylation by Exon Splicing

INTRODUCTION

In GP disease, the immune system attack is mediated by autoantibodies against the non-collagenous C-terminal domain (NC1) of the α3 chain of collagen IV (the GP antigen) (1). The N-terminus of the human α3(IV)NC1 contains a highly divergent and hydrophilic region with a unique structural motif, $KRGDS^9$, that harbors a cell adhesion signal as an integral part of a functional phosphorylation site for type A protein kinases (2,3). Furthermore, the gene region encoding the human GP antigen characteristically generates multiple mRNAs by alternative exon splicing (4,5). The alternative products diverge in the C-terminal ends and all but one share the N-terminal $KRGDS^9$ (4,5).

Multiple sclerosis (MS) is an exclusive human neurological disease characterized by the presence of inflamatory demyelization plaques at the central nervous system. (6). Several evidences indicate that this disease is caused by an autoimmune attack mediated by cytotoxic T cells towards specific components of the white matter including the myelin

basic protein (MBP) (7, 8). In humans, the MBP gene generates four products (MBP, MBPΔII, MBPΔV and MBPΔII/V) that result from alternative exon splicing during pre-mRNA processing (9). Among these, MBPΔII is the more abundant form in the mature central nervous system, while MBP form containing all the exons is virtually absent (9).

Several biological similarities exist between the autoimme responses mediating GP disease and MS, namely: 1) both are human exclusive diseases and typically initiate after a viral flu-like disease; 2) a strong linkage exists to the same haplotype of the HLA-DR region of the class II MHC; 3) several products are generated by alternative splicing; and 4) the death of a MS patient by GP disease has recently been reported (10).

MATERIALS AND METHODS

Synthetic polymers: GPΔIII derived peptide, QRAHGQDLDALFVKVLRSP (SEQ ID NO:43) and GPΔIII/IV/V derived peptide, QRAHGQDLESLFHQL (SEQ ID NO:44) were synthesized using either Boc- (MedProbe) or Fmoc- (Chiron, Lipotec) chemistry.

Plasmid construction and recombinant expression.

GP derived material: The constructs representing the different GP-spliced forms were obtained by subcloning the cDNAs used elsewhere to express the corresponding recombinant proteins (5) into the BamHI site of a modified pET15b vector, in which the extraneous vector-derived amino-terminal sequence except for the initiation Met was eliminated. The extra sequence was removed by cutting the vector with NcoI and Bam HI, filling-in of the free ends with Klenow, and re-ligation. This resulted in the reformation of both restriction sites and placed the BamHI site immediately downstream of the codon for the amino-terminal Met.

The recombinant proteins representing GP or GPΔV (SEQ ID NO:46) were purified by precipitation (5). Bacterial pellets containing the recombinant proteins representing GPΔIII (SEQ ID NO:48) or GPΔIII/IV/V (SEQ ID NO:50) were dissolved by 8 M urea in 40 mM Tris-HCl pH 6.8 and sonication. After centrifugation at 40,000 x *g* the supernatants were passed through a 0.22 μm filter and applied to resource Q column for FPLC. The effluent was acidified to pH 6 with HCl and applied to a resource S column previously equilibrated with 40 mM MES pH 6 for a second FPLC

purification. The material in the resulting effluent was used for in vitro phosphorylation.

MBP-derived material: cDNA representing human MBPΔII (SEQ ID NO:51) was obtained by RT-PCR using total RNA from central nervous system. The cDNA representing human MBP was a generous gift from C. Campagnoni (UCLA). Both fragments were cloned into a modified version of pHIL-D2 (Invitrogen) containing a 6xHis-coding sequence at the C-terminus to generate pHIL-MBPΔII-His and pHIL-MBP-His, respectively. These plasmids were used for recombinant expression in *Pichia pastoris* as described in (12). Recombinant proteins were purified using immobilized metal affinity chromatography (TALON resin, CLONTECH) under denaturant conditions (8M urea) and eluted with 300 mM imidazole following manufacturers' instructions. The affinity-purified material was then renatured by dilution into 80 volumes of 50 mM Tris-HCl pH 8.0, 10 mM CHAPS, 400 mM NaCl, 2 mM DTT, and concentrated 50 times by ultrafiltration through a YM10-type membrane (AMICON). The Ser to Ala mutants were produced by site-directed mutagenesis over native sequence-containing constructs using transformer mutagenesis kit from CLONTECH and the resulting proteins were similarly produced.

Phosphorylation studies. Phosphorylation studies were essentially done as described above (see also 3 and 12). In some experiments, the substrates were in-blot renatured and then, phosphorylated for 30 min at room temperature by overlaying 100 µl of phosphorylation buffer containing 0.5 µg of rGPBP. Digestion with V8 endopeptidase and immunoprecipitation were performed as described in (3).

Antibody production. Synthetic peptides representing the C-terminal divergent ends of GPΔIII or GPΔIII/IV/V comprised in SEQ ID NO:43 or SEQ ID NO:44 respectively were conjugated to a cytochrome C, BSA or ovoalbumine using a glutaraldehyde coupling standard procedure. The resulting protein conjugates were used for mouse immmunization to obtain polyclonal antibodies specific for GPΔIII and monoclonal antibodies specific for GPΔIII/IV/V (Mab153). To obtain monoclonal antibodies specific for GPΔV (Mab5A) mouse were immunized using recombinant bacterial protein representing the corresponding alternative form comprising the SEQ ID NO:50. The production of monoclonal (M3/1, P1/2) or polyclonal (anti-GPpep1)

antibodies against SEQ ID NO: 26 which represents the N-terminal region of the GP alternative forms have been previously described (3,5).

Boc-based peptide synthesis.

Assembling .The peptide was assembled by stepwise solid phase synthesis using a Boc-Benzyl strategy. The starting resin used was Boc-Pro-PAM resin (0.56 meq/g, batch R4108). The deprotection /coupling procedure used was: TFA (1x1min) TFA (1x 3 min) DCM (flow flash) Isopropylalcohol (1x 30 sec) DMF (3 x 1 min) COUPLING/DMF (1 x10 min) DMF (1x1 min) COUPLING/DMF (1x 10 min) DMF (2x 1min) DCM (1x 1min). For each step 10 ml per gram of peptide-resin were used. The coupling of all amino acids (fivefold excess) was performed in DMF in the presence of BOP, Hobt and DIEA. For the synthesis the following side-chain protecting groups were used: benzyl for serine; 2 chlorobenzyloxycarbonyl for lysine; cyclohexyl for aspartic and glutamic acid; tosyl for histidine and arginine.

Cleavage. The peptide was cleaved from the resin and fully deprotected by a treatment with liquid Hydrogen Fluoride (HF): Ten milliliters of HF per gram of peptide resin were added and the mixture kept at 0° C for 45 min in the presence of p-cresol as scavengers. After evaporation of the HF, the crude reaction mixture is washed with ether, dissolved in TFA, precipitated with ether and dried.

Purification. Stationary phase: Silica C18, 15 μm, 120 A; Mobile phase: solvent A: water 0.1% TFA and solvent B: acetonitrile /A, 60/40 (v/v); Gradient: linear from 20 to 60% B in 30 min; Flow rate: 40 ml/min; and detection was U.V (210 nm). Fractions with a purity higher than 80% were pooled and lyophilized. Control of purity and identity was performed by analytical HPLC and ES/MS. The final product had 88% purity and an experimental molecular weight of 2192.9.

Fmoc-based peptide synthesis.

Assembling. The peptides were synthesized by stepwise linear solid phase on Pro-clorotrityl-resin (0.685 meq/g) with standard Fmoc/tBu chemistry. The deprotection /coupling procedure used was: Fmoc aa (0.66 g) HOBt (0.26 g) DIPCDI (0.28 ml) for 40 min following a control by Kaiser test. If the test was positive the time was extended until change to negative. Then DMF (31 min), piperidine/DMF 20% (11 min) piperidine/DMF 20% (15 min) and DMF (41 min). Side chain protectors were:

Pmc (pentamethylcromane sulfonyl) for arginine, Bcc (tert-butoxycarbonyl) for lysine, tBu (tert-butyl) for aspartic acid and for serine and Trl (trityl) for histidine.

Cleavage. The peptide was cleaved and fully deprotected by treatment cleavage with TFA/water 90/10. Ten milliliters of TFA solution per gram of resin were added. Water acts as scavenger. After two hours, resin was filtered and the resulting solution was precipitated five times with cold diethylether. The final precipitated was dried.

Purification. Stationary phase: Kromasil C18 10 μm; Mobile phase: solvent A: water 0.1% TFA and solvent B: acetonitrile 0.1% TFA; Isocratic: 28% B; Flow rate: 55 ml/min; Detection: 220 nm. Fractions with the higher purity were pooled and lyophilized, and a second HPLC purification round performed. Control of purity and identity was performed by analytical HPLC and ES/MS. Thc final product had 97% purity and an experimental molecular weight of 2190.9.

RESULTS

Regulation of the phosphorylation of the human GP antigen by alternative splicing. We produced bacterial recombinant proteins representing the primary antigen (GP) or the individual alternative products GPΔV (SEQ ID NO:46), GPΔIII (SEQ ID NO:48) and GPΔIII/IV/V (SEQ ID NO:50), and we tested their ability to be phosphorylated by PKA (Figure 16, left panel). Using standard ATP concentrations (150 μM), all four recombinant antigens were phosphorylated but to very different extents. The alternative forms incorporated ^{32}P more efficiently than the primary GP antigen, suggesting that they are better substrates. Because these antigens are expected to be in the extracellular compartment, we also assayed their phosphorylatability with more physiological ATP concentrations (0.1-0.5 μM). Under these conditions, the differences in ^{32}P incorporation between the primary and alternative products were more evident, indicating that at low ATP concentrations the primary GP antigen was a very poor substrate for the kinase. Among the three PKA phosphorylation sites present in the GP antigen, the N-terminal Ser^{9} and Ser^{26} are the major ones, and are common to all the alternative products assayed (3,5). Accordingly, the differences observed in phosphorylation for the full polypeptides also existed among the individual N-terminal regions, as determined after specific V8 digestion and immunoprecipitation (not shown). This strongly suggests that differences in phosphorylation might be due to the presence of

different C-terminal sequences in the alternative products. Since GPΔIII and GPΔIII/IV/V displayed significantly higher ^{32}P incorporation rates than GPΔV, and they have shorter divergent C-terminal regions (5), we used synthetic peptides individually representing these C-terminal sequences (SEQ ID NO: 43, SEQ ID NO:44) to further examine their regulatory roles in the in vitro phosphorylation of the native antigen. Collagen IV is a trimeric molecule comprised of three interwoven α chains. In basement membranes, two collagen IV molecules assemble through their NC1 domains to yield a hexameric NC1 structure that can be solubilized by bacterial collagenase digestion (1). Dissociation of the hexamer structure releases the GP antigen in monomeric and disulfide-related dimeric forms (1). For the following set of experiments, we carried out phosphorylations in the presence of low, extracellular-like ATP concentrations using both monomeric or hexameric native GP antigen (Figure 16, right panel). The presence of each specific peptide but not control peptides (not shown) induced the phosphorylation of a single polypeptide displaying an apparent MW of 22 kDa. By specific V8 digestion and immunoprecipitation, the corresponding polypeptide has been identified as the 22 kDa conformer of the α3(IV)NC1, previously characterized and identified as the best substrate for the PKA (11).

Regulation of the phosphorylation of the MBP by alternative splicing. The MBP contains at its N terminal region two PKA phosphorylation sites (Ser^8, Ser^{57}) that are structurally similar to the N terminus site (Ser^9) present in GP antigen products (Fig 17). The Ser^8 site present in all the MBP proteins is located in a similar position than the Ser^9 in the GP-derived polypeptides. In addition, in the MBP and GPΔIII Ser^8 and Ser^9 respectively are at a similar distance in the primary structures of a highly homologous motif present in the corresponding exon II (bend arrow in Fig 17). The GPΔIII-derived motif coincides with the C terminal divergent region that up-regulates PKA phosphorylation of Ser^9 in the GP antigen system (Fig. 16). The regulatory-like sequence in MBP is located at exon II and its presence in the final products depends on an alternative exon splicing mechanism. Therefore, the MBP motif identified by structural comparison to GPΔIII may be also regulating PKA phosphorylation of Ser^8. We produced recombinant proteins representing MBP and MBPΔII (SEQ ID NO:54) and the corresponding Ser to Ala mutants to knock-out each of the two PKA phosphorylation sites (Ser^8 and Ser^{57}) present in exon I. Subsequently, we assessed its in vitro phosphorylation

by PKA (Fig. 18). MBPΔII was a better substrate than MBP, and Ser^8 was the major phosphorylation site, indicating that, similarly to GP antigenic system, alternative exon splicing regulates the PKA phosphorylation of specific sites located at the N-terminal region common to all the MBP-derived alternative forms.

In similar experiments assessing GPBP phosphorylation of the recombinant MBP proteins, GPBP preferentially phosphorylated MBP, while little phosphorylation of MBPΔII was observed (Fig. 19). Furthermore, recombinant Ser to Ala mutants displayed no significant reduction in ^{32}P incorporation, indicating that GPBP phosphorylates MBP/MBPΔII in an opposite way than PKA, and that these two kinases do not share major phosphorylation sites in MBP proteins.

From all these data we concluded that in the MBP system, alternative splicing regulates the phosphorylation of specific serines by either PKA or GPBP.

Synthetic peptides representing the C terminal region of GPΔIII influence GPBP phosphorylation. To assess the effect of the C terminal region of GPΔIII on GPBP activity, peptides representing this region were synthesized using two different chemistries (Boc or Fmoc), and separately added to a phosphorylation mixture containing GPBP (Fig. 20). Boc-based synthetic peptides positively influenced GPBP autophosphorylation while Fmoc-based inhibited GPBP autophosphorylation, suggesting that the regulatory sequences derived from the alternative products in either GP and MBP antigenic systems can influence the kinase activity of GPBP.

DISCUSSION

We have shown that the α3(IV)NC1 domain undergoes a complex structural diversification by two different mechanism: 1) alternative splicing (4,5) and 2) conformational isomerization of the primary product (11). Both mechanisms generate products that are distinguished by PKA, indicating that PKA phosphorylation is a critical event in the biology of the α3(IV)NC1 domain. Phosphorylation guides at least in part the folding, but also the supramolecular assembly of the α3(IV)NC1 domain in the collagen IV network (11 and Raya et al. unpublished results). Altered conformers of the α3(IV)NC1 lead the autoimmune response mediating GP disease (11), suggesting that an alteration in antigen phosphorylation could be the primary event in the onset of

the disease. Accordingly, we have found increased expression levels of GPΔIII in several GP kidneys (4 and Bernal and Saus, unpublished results), and an increased expression of GPBP has been detected in another Goodpasture patient (Fig. 15). Both increased expression of alternative GP antigen products and of GPBP are expected to have consequences in the phosphorylation steady state of α3(IV)NC1, and therefore in the corresponding conformational process. The discrimination among the different structural products by PKA strongly suggests that this kinase, or another structurally similar kinase, is involved in the physiological antigen conforming process, and that antigen phosphorylation by GPBP has a pathogenic significance. In pathogenesis, GPBP could be an intruding kinase, interfering in the phosphorylation-dependent conforming process. Accordingly, GPBP is expressed in tissue structures that are targeted by natural autoimmune responses, and an increased expression of GPBP is associated with several autoimmune conditions (See examples 1 and 2 above).

An alternative splicing mechanism also regulates the PKA phosphorylation of specific serines in the MBP antigenic system. MBP is also a substrate for GPBP suggesting that GPBP may play a pathogenic role in multiple sclerosis, and other autoimmune responses.

All of the above data identify GPBP as a potential target for therapeutics in autoimmune disease. In Fig 20, we show that synthetic peptides representing the C terminal region of GPΔIII (SEQ ID NO:43) modulate the action of GPBP in vitro, and therefore we identified this and related sequences as peptide-based compounds to modulate the activity of GPBP in vivo. The induction of GP antigen phosphorylation by PKA was achieved when using Boc-based peptides, but not when using similar Fmoc-based peptides. Furthermore, Boc- but not Fmoc-based peptides were in vitro substrates of PKA (not shown), indicating that important structural differences exist between both products. Since both products displayed no significant differences in mass spectrometry, one possibility is that the different deprotection procedure used may be responsible for conformational differences in the secondary structure that may be critical for biological activity. Accordingly, Boc-based peptide loses its ability to induce PKA upon long storage at low temperatures.

REFERENCES FOR EXAMPLE 3

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The present invention is not limited by the aforementioned particular preferred embodiments. It will occur to those ordinarily skilled in the art that various modifications may be made to the disclosed preferred embodiments without diverting from the concept of the invention. All such modifications are intended to be within the scope of the present invention.

I claim:

1. An isolated nucleic acid sequence comprising a sequence substantially similar to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25.

2. An isolated nucleic acid sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25.

3. An isolated nucleic acid comprising a sequence that encodes a polypeptide selected from the group consisting of GPBP, GPBPΔ26, and GPBPpep1, or fragments thereof.

4. An isolated nucleic acid sequence comprising a sequence that encodes a protein sequence substantially similar to a protein sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24

5. An isolated nucleic acid sequence comprising a sequence that encodes a protein sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24.

6. A recombinant expression vector comprising the isolated nucleic acid sequence of any one of claims 1-5.

7. A recombinant expression vector comprising an isolated nucleic acid sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25, or fragments thereof

8. A host cell transfected with the recombinant expression vector of claim 6 or 7.

9. A substantially purified polypeptide, comprising an amino acid sequence substantially similar to a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or peptide fragments thereof

10. A substantially purified polypeptide, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or peptide fragments thereof.

11. A substantially purified protein comprising a polypeptide selected from the group consisting of GPBP, GPBPΔ26, and GPBPpep1, or peptide fragments thereof.

12. An antibody that selectively binds to the substantially purified protein or polypeptide of any one of claims 9-11.

13. The antibody of claim 12, wherein the antibody is a polyclonal antibody.

14. The antibody of claim 12, wherein the antibody is a monoclonal antibody.

15. A method for detecting the presence of a protein that is substantially similar to a protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID

NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, comprising

a) providing a protein sample to be screened;

b) contacting the protein sample to be screened with the antibody of any one of claims 12-14 under conditions that promote antibody-antigen complex formation; and

c) detecting the formation of antibody-antigen complexes, wherein the presence of the antibody-antigen complex indicates the presence of a protein that is substantially similar to a protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24.

16. The method of claim 15, wherein detecting comprises a method selected from the group consisting of immunolocalization, immunofluorescence analysis, Western blot analysis, ELISAs, and nucleic acid expression library screening.

17. A method for detecting in a sample a sequence that is substantially similar to a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25, comprising contacting the sample with the isolated nucleic acid of any one of claims 1-5, or fragments thereof, and detecting complex formation, wherein complex formation indicates the presence in the sample of the sequence that is substantially similar to a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:25.

18. The method of claim 17, wherein the detecting is carried out by a method selected from the group consisting of hybridization, reverse transcription, PCR, coupled reverse transcription-PCR, Northern blotting, Southern blotting, and DNA library screening.

19. A method for detecting an autoimmune condition in a patient, comprising

-providing a tissue or body fluid sample from the patient;

-providing a control tissue or body fluid sample in which no autoimmune condition is present; and

-detecting altered GPBP RNA or protein expression in the tissue or body fluid sample compared to the control sample, wherein an alteration in GPBP RNA or protein expression relative to the control indicates the presence of an autoimmune condition.

20. A method for detecting cells undergoing apoptosis or cancer transformation in a tissue or body fluid sample, comprising

-providing a tissue or body fluid sample from the patient;

-providing a normal control tissue or body fluid sample; and

-detecting altered GPBP RNA or protein expression in the tissue or body fluid sample compared to the control sample, wherein an alteration in GPBP RNA or protein expression relative to the control indicates the presence of cells undergoing apoptosis or cancer transformation.

21. A method for treating a patient with an autoimmune disorder, comprising modifying the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1 in the patient with the autoimmune disorder.

22. A method for treating a patient with a tumor, comprising modifying the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1 in the patient with the tumor.

23. A method for preventing cell apoptosis, comprising modifying the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1 in the cell.

24. The method of claim 21, 22, or 23 wherein alternative products of the Goodpasture antigen or of the myelin basic protein are used to modify the expression or activity of GPBP, GPBPΔ26 or a protein comprising a polypeptide substantially similarly to GPBPpep1.

25. The method of claim 21, 22, or 23 wherein nucleic acids comprising sequences substantially similar to SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO: 51, or SEQ ID NO:53 or fragments thereof are used to modify the expression or activity of GPBP, GPBPΔ26 or a protein comprising a polypeptide substantially similarly to GPBPpep1.

26. The method of claim 21, 22, or 23 wherein polypeptides comprising sequences substantially similar to SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, or SEQ ID NO:54, or fragments thereof are used to modify the expression or activity of GPBP, GPBPΔ26 or a protein comprising a polypeptide substantially similarly to GPBPpep1.

27. An isolated nucleic acid sequence comprising a sequence that encodes a polypeptide substantially similar to an amino acid sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, or peptide fragments thereof.

28. An isolated nucleic acid sequence comprising a sequence that encodes a polypeptide selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, and peptide fragments thereof.

29. A recombinant expression vector comprising the isolated nucleic acid sequence of claim 27 or 28.

30. A host cell transfected with the recombinant expression vector of claim 29.

31. A substantially purified polypeptide, comprising an amino acid sequence substantially similar to a sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, or peptide fragments thereof

32. A substantially purified polypeptide, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, or peptide fragments thereof.

33. An antibody that selectively binds to the substantially purified protein or polypeptide of claim 31 or 32.

34. The antibody of claim 33, wherein the antibody is a polyclonal antibody.

35. The antibody of claim 33, wherein the antibody is a monoclonal antibody.

36. The method of claim 21, 22, or 23 comprising administering a substantially purified polypeptide substantially similar to a polypeptide selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, or SEQ ID NO:54, or fragments thereof, to modify the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1.

37. The method of claim 21, 22, or 23 comprising administering an isolated nucleic acid comprising sequences substantially similar to SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO: 51, or SEQ ID NO:53 or fragments thereof, or fragments thereof, to modify the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1.

38. A pharmaceutical composition, comprising an amount effective of a substantially purified polypeptide substantially similar to a polypeptide selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, or SEQ ID NO:54, or fragments thereof, to modify the

expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1, and a pharmaceutically acceptable carrier.

39. A pharmaceutical composition, comprising an amount effective of a an isolated nucleic acid comprising sequences substantially similar to SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO: 51, or SEQ ID NO:53 or fragments thereof, to modify the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1, and a pharmaceutically acceptable carrier.

40. The method of claim 21, 22, or 23 comprising administering the pharmaceutical composition of claim 38 or 39 to modify the expression or activity of GPBP, GPBPΔ26, or a protein comprising a polypeptide substantially similarly to GPBPpep1.

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GCAGGAAGATGGCGGCGGTAGCGGAGGTGTGAGTGGACGCGGGACTCAGCGGCCGGATTTTCTCTTCCCT   70

TCTTTTCCCTTTTCCTTCCCTATTTGAAATTGGCATCGAGGGGGCTAAGTTCGGGTGGCAGCGCCGGGCG  140

CAACGCAGGGGTCACGGCGACGGCGGCGGCGGCTGACGGCTGGAAGGGTAGGCTTCATTCACCGCTCGTC  210

CTCCTTCCTCGCTCCGCTCGGTGTCAGGCGCGGCGGCGGCGCGGCGGGCGGACTTCGTCCCTCCTCCTGC  280

TCCCCCCCACACCGGAGCGGGCACTCTTCGCTTCGCCATCCCCCGACCCTTCACCCCGAGGACTGGGCGC  350

CTCCTCCGGCGCAGCTGAGGGAGCGGGGGCCGGTCTCCTGCTCGGTTGTCGAGCCTCCATGTCGGATAAT  420
                                                        M  S  D  N             4

CAGAGCTGGAACTCGTCGGGCTCGGAGGAGGATCCAGAGACGGAGTCTGGGCCGCCTGTGGAGCGCTGCG  490
 Q  S  W  N  S  S  G  S  E  E  D  P  E  T  E  S  G  P  P  V  E  R  C      27

GGGTCCTCAGTAAGTGGACAAACTACATTCATGGGTGGCAGGATCGTTGGGTAGTTTTGAAAAATAATGC  560
G  V  L  S  K  W  T  N  Y  I  H  G  W  Q  D  R  W  V  V  L  K  N  N  A     51

TCTGAGTTACTACAAATCTGAAGATGAAACAGAGTATGGCTGCAGAGGATCCATCTGTCTTAGCAAGGCT  630
   L  S  Y  Y  K  S  E  D  E  T  E  Y  G  C  R  G  S  I  C  L  S  K  A     74

GTCATCACACCTCACGATTTTGATGAATGTCGATTTGATATTAGTGTAAATGATAGTGTTTGGTATCTTC  700
 V  I  T  P  H  D  F  D  E  C  R  F  D  I  S  V  N  D  S  V  W  Y  L       97

GTGCTCAGGATCCAGATCATAGACAGCAATGGATAGATGCCATTGAACAGCACAAGACTGAATCTGGATA  770
R  A  Q  D  P  D  H  R  Q  Q  W  I  D  A  I  E  Q  H  K  T  E  S  G  Y    121

TGGATCTGAATCCAGCTTGCGTCGACATGGCTCAATGGTGTCCCTGGTGTCTGGAGCAAGTGGCTACTCT  840
   G  S  E  S  S  L  R  R  H  G  S  M  V  S  L  V  S  G  A  S  G  Y  S    144

GCAACATCCACCTCTTCATTCAAGAAAGGCCACAGTTTACGTGAGAAGTTGGCTGAAATGGAAACATTTA  910
 A  T  S  T  S  S  F  K  K  G  H  S  L  R  E  K  L  A  E  M  E  T  F      167

GAGACATCTTATGTAGACAAGTTGACACGCTACAGAAGTACTTTGATGCCTGTGCTGATGCTGTCTCTAA  980
R  D  I  L  C  R  Q  V  D  T  L  Q  K  Y  F  D  A  C  A  D  A  V  S  K    191

GGATGAACTTCAAAGGGATAAAGTGGTAGAAGATGATGAAGATGACTTTCCTACAACGCGTTCTGATGGT 1050
   D  E  L  Q  R  D  K  V  V  E  D  D  E  D  D  F  P  T  T  R  S  D  G    214

GACTTCTTGCATAGTACCAACGGCAATAAAGAAAAGTTATTTCCACATGTGACACCAAAAGGAATTAATG 1120
 D  F  L  H  S  T  N  G  N  K  E  K  L  F  P  H  V  T  P  K  G  I  N      237

GTATAGACTTTAAAGGGGAAGCGATAACTTTTAAAGCAACTACTGCTGGAATCCTTGCAACACTTTCTCA 1190
G  I  D  F  K  G  E  A  I  T  F  K  A  T  T  A  G  I  L  A  T  L  S  H    261

TTGTATTGAACTAATGGTTAAACGTGAGGACAGCTGGCAGAAGAGACTGGATAAGGAAACTGAGAAGAAA 1260
   C  I  E  L  M  V  K  R  E  D  S  W  Q  K  R  L  D  K  E  T  E  K  K    284

AGAAGAACAGAGGAAGCATATAAAAATGCAATGACAGAACTTAAGAAAAAATCCCACTTTGGAGGACCAG 1330
 R  R  T  E  E  A  Y  K  N  A  M  T  E  L  K  K  K  S  H  F  G  G  P      307

ATTATGAAGAAGGCCCTAACAGTCTGATTAATGAAGAAGAGTTCTTTGATGCTGTTGAAGCTGCTCTTGA 1400
D  Y  E  E  G  P  N  S  L  I  N  E  E  E  F  F  D  A  V  E  A  A  L  D    331
```

FIG. 1

```
CAGACAAGATAAAATAGAAGAACAGTCACAGAGTGAAAAGGTGAGATTACATTGGCCTACATCCTTGCCC 1470
  R  Q  D  K  I  E  E  Q  S  Q  S  E  K  V  R  L  H  W  P  T  S  L  P   354

TCTGGAGATGCCTTTTCTTCTGTGGGGACACATAGATTTGTCCAAAAGCCCTATAGTCGCTCTTCCTCCA 1540
  S  G  D  A  F  S  S  V  G  T  H  R  F  V  Q  K  P  Y  S  R  S  S  S   377

TGTCTTCCATTGATCTAGTCAGTGCCTCTGATGATGTTCACAGATTCAGCTCCCAGGTTGAAGAGATGGT 1610
  M  S  S  I  D  L  V  S  A  S  D  D  V  H  R  F  S  S  Q  V  E  E  M  V   401

GCAGAACCACATGACTTACTCATTACAGGATGTAGGCGGAGATGCCAATTGGCAGTTGGTTGTAGAAGAA 1680
  Q  N  H  M  T  Y  S  L  Q  D  V  G  G  D  A  N  W  Q  L  V  V  E  E   424

GGAGAAATGAAGGTATACAGAAGAGAAGTAGAAGAAAATGGGATTGTTCTGGATCCTTTAAAAGCTACCC 1750
  G  E  M  K  V  Y  R  R  E  V  E  E  N  G  I  V  L  D  P  L  K  A  T   447

ATGCAGTTAAAGGCGTCACAGGACATGAAGTCTGCAATTATTTCTGGAATGTTGACGTTCGCAATGACTG 1820
H  A  V  K  G  V  T  G  H  E  V  C  N  Y  F  W  N  V  D  V  R  N  D  W   471

GGAAACAACTATAGAAAACTTTCATGTGGTGGAAACATTAGCTGATAATGCAATCATCATTTATCAAACA 1890
  E  T  T  I  E  N  F  H  V  V  E  T  L  A  D  N  A  I  I  I  Y  Q  T   494

CACAAGAGGGTGTGGCCTGCTTCTCAGCGAGACGTATTATATCTTTCTGTCATTCGAAAGATACCAGCCT 1960
  H  K  R  V  W  P  A  S  Q  R  D  V  L  Y  L  S  V  I  R  K  I  P  A   517

TGACTGAAAATGACCCTGAAACTTGGATAGTTTGTAATTTTTCTGTGGATCATGACAGTGCTCCTCTAAA 2030
L  T  E  N  D  P  E  T  W  I  V  C  N  F  S  V  D  H  D  S  A  P  L  N   541

CAACCGATGTGTCCGTGCCAAAATAAATGTTGCTATGATTTGTCAAACCTTGGTAAGCCCACCAGAGGGA 2100
  N  R  C  V  R  A  K  I  N  V  A  M  I  C  Q  T  L  V  S  P  P  E  G   564

AACCAGGAAATTAGCAGGGACAACATTCTATGCAAGATTACATATGTAGCTAATGTGAACCCTGGAGGAT 2170
  N  Q  E  I  S  R  D  N  I  L  C  K  I  T  Y  V  A  N  V  N  P  G  G   587

GGGCACCAGCCTCAGTGTTAAGGGCAGTGGCAAAGCGAGAGTATCCTAAATTTCTAAAACGTTTTACTTC 2240
W  A  P  A  S  V  L  R  A  V  A  K  R  E  Y  P  K  F  L  K  R  F  T  S   611

TTACGTCCAAGAAAAAACTGCAGGAAAGCCTATTTTGTTCTAGTATTAACAGGTACTAGAAGATATGTTT 2310
  Y  V  Q  E  K  T  A  G  K  P  I  L  F                                   624

TATCTTTTTTTAACTTTATTTGACTAATATGACTGTCAATACTAAAATTTAGTTGTTGAAAGTATTTACT 2380

ATGTTTTTT                                                               2389
```

FIG. 1

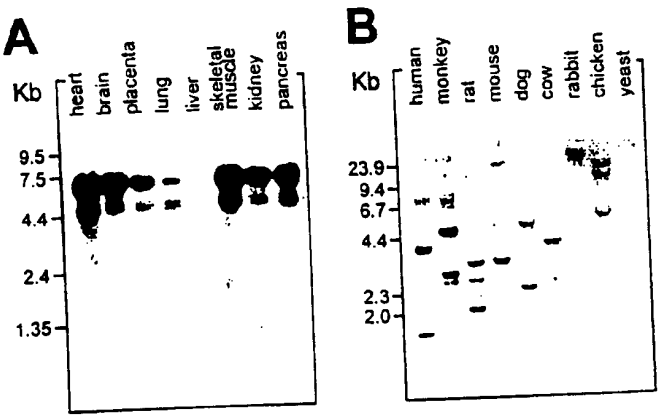

A
Kb
heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
9.5
7.5
4.4
2.4
1.35
B
Kb
human
monkey
rat
mouse
dog
cow
rabbit
chicken
yeast
23.9
9.4
6.7
4.4
2.3
2.0

FIG. 2

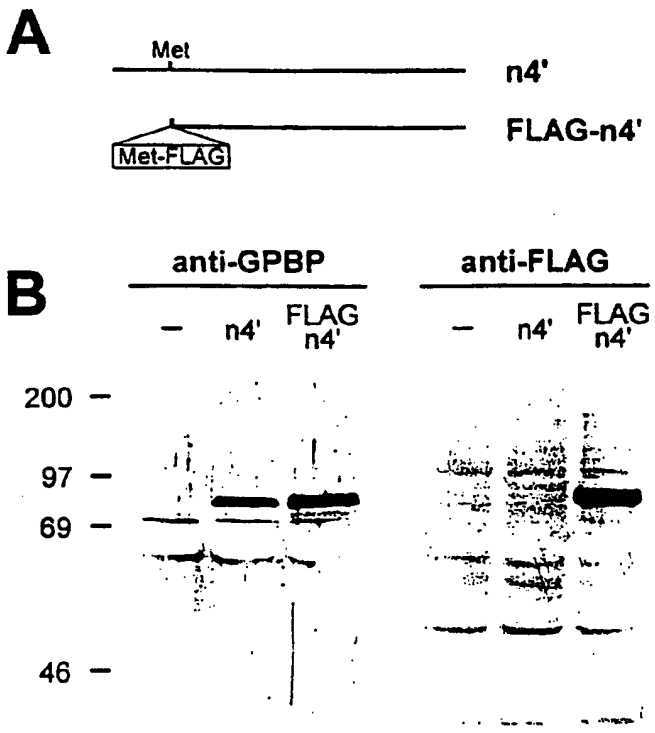

A
Met
n4'
FLAG-n4'
Met-FLAG
B
anti-GPBP
anti-FLAG
–
n4'
FLAG n4'
200
97
69
46

FIG. 3

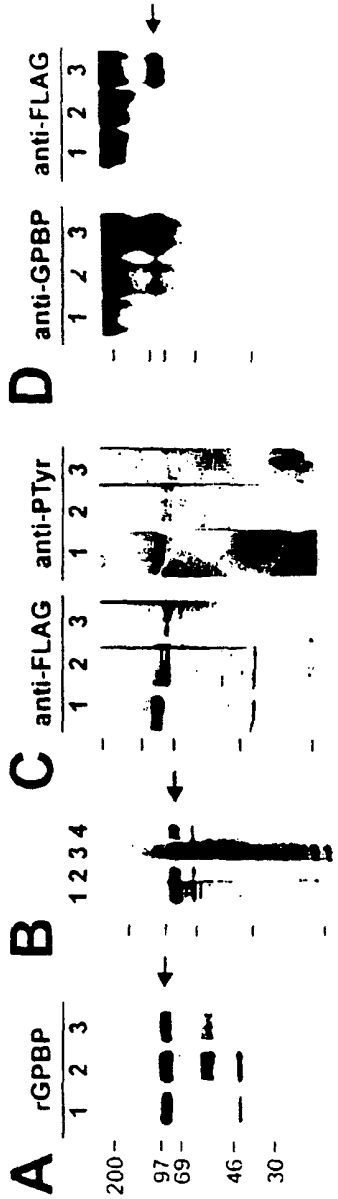

A
rGPBP
1 2 3
200
97
69
46
30
B
1 2 3 4
C
anti-FLAG
1 2 3
anti-PTyr
1 2 3
D
anti-GPBP
1 2 3
anti-FLAG
1 2 3

FIG. 4

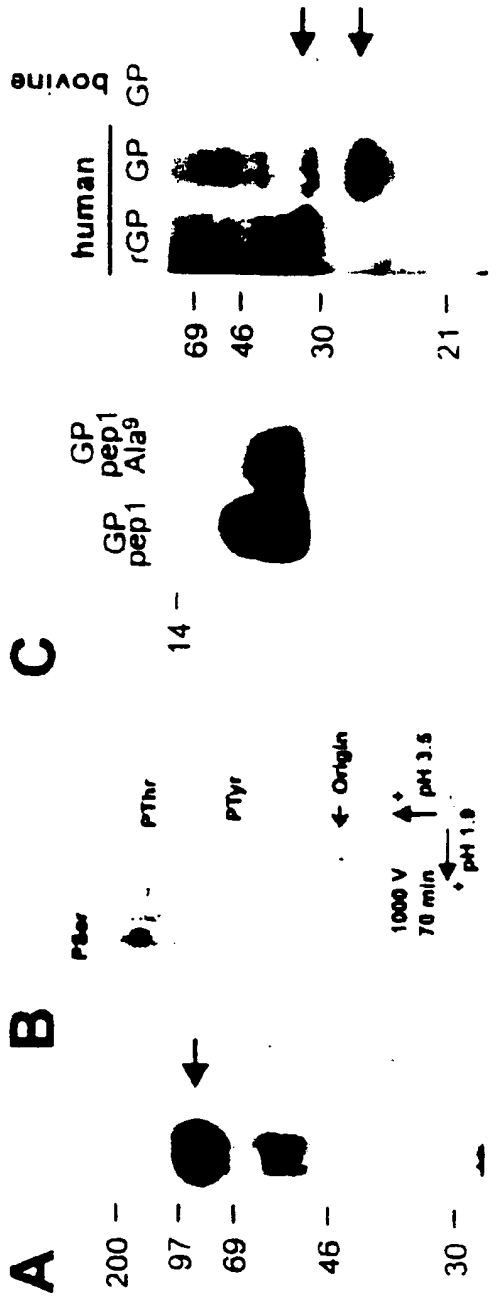

A
200 –
97 –
69 –
46 –
30 –
B
PSer
PThr
PTyr
Origin
1000 V
70 min
pH 3.5
pH 1.9
C
GP pep1
GP pep1 Ala9
14 –
human
rGP
GP
bovine
GP
69 –
46 –
30 –
21 –

FIG. 5

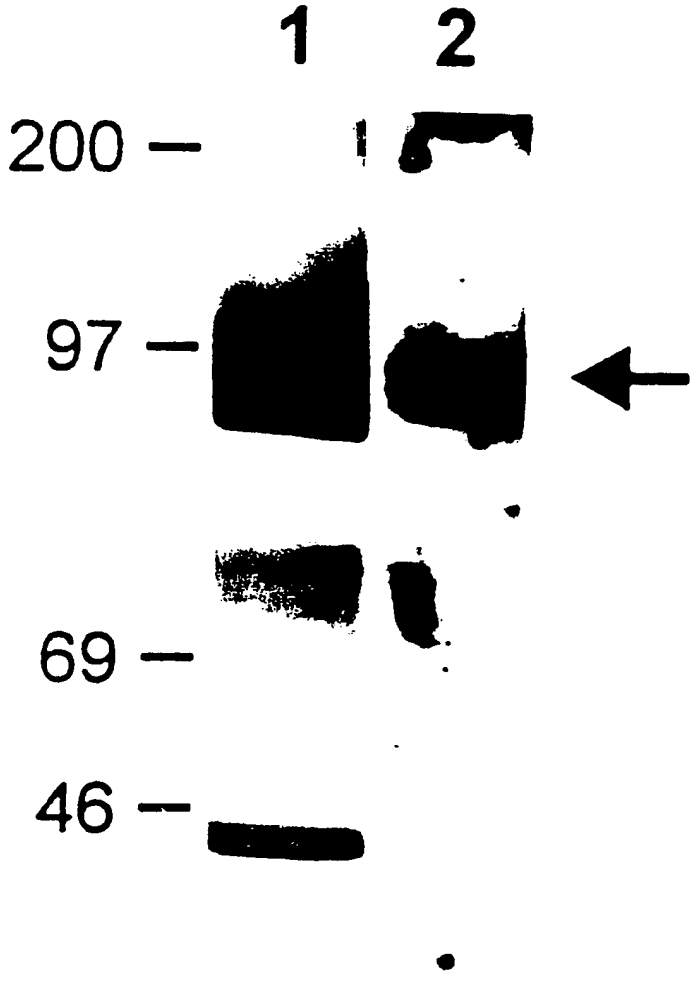


FIG. 6

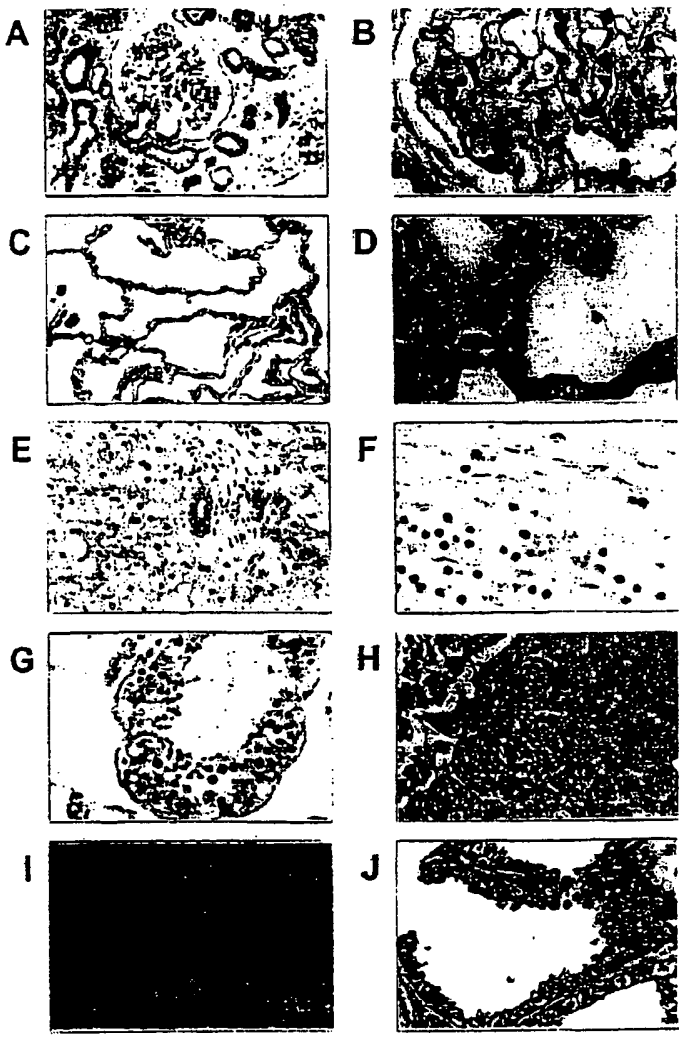
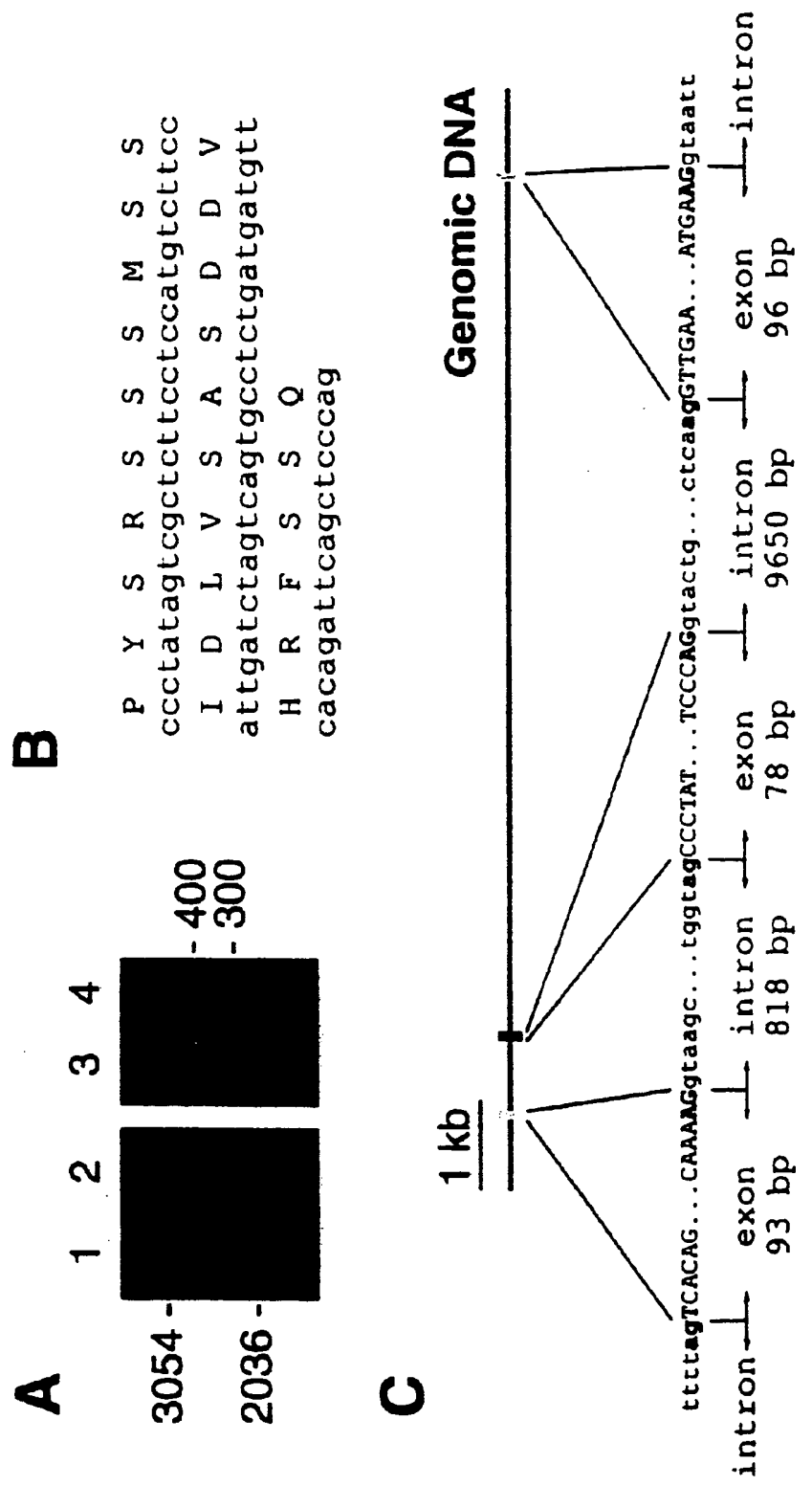


FIG. 7


A
1 2 3 4
3054 -
2036 -
- 400
- 300
B
P Y S R S S S M S S
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I D L V S A S D D V
attgatctagtcagtgcctctgatgatgtt
H R F S S Q
cacagattcagctcccag
C
1 kb
Genomic DNA
intron
exon
93 bp
intron
818 bp
exon
78 bp
intron
9650 bp
exon
96 bp
intron
ttttagTCACAG...CAAAGgtaagc....tggtagCCCTAT...TCCCAGgtactg....ctcaagGTTGAA...ATGAAGgtaatt

FIG. 8

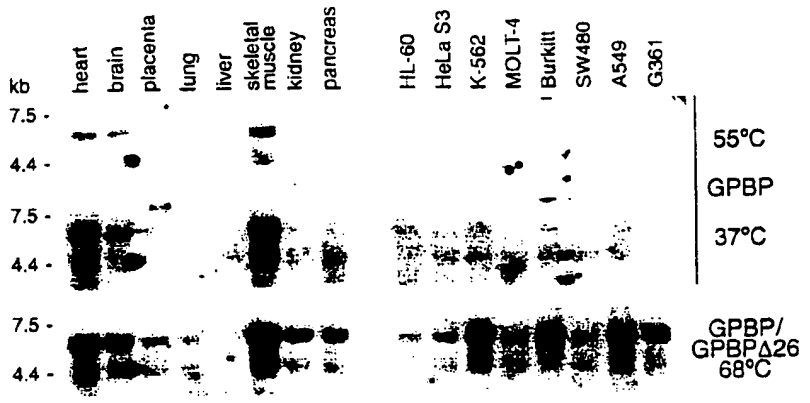

kb
heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
HL-60
HeLa S3
K-562
MOLT-4
Burkitt
SW480
A549
G361
7.5 -
4.4 -
7.5 -
4.4 -
7.5 -
4.4 -
55°C
GPBP
37°C
GPBP/
GPBPΔ26
68°C

FIG. 9

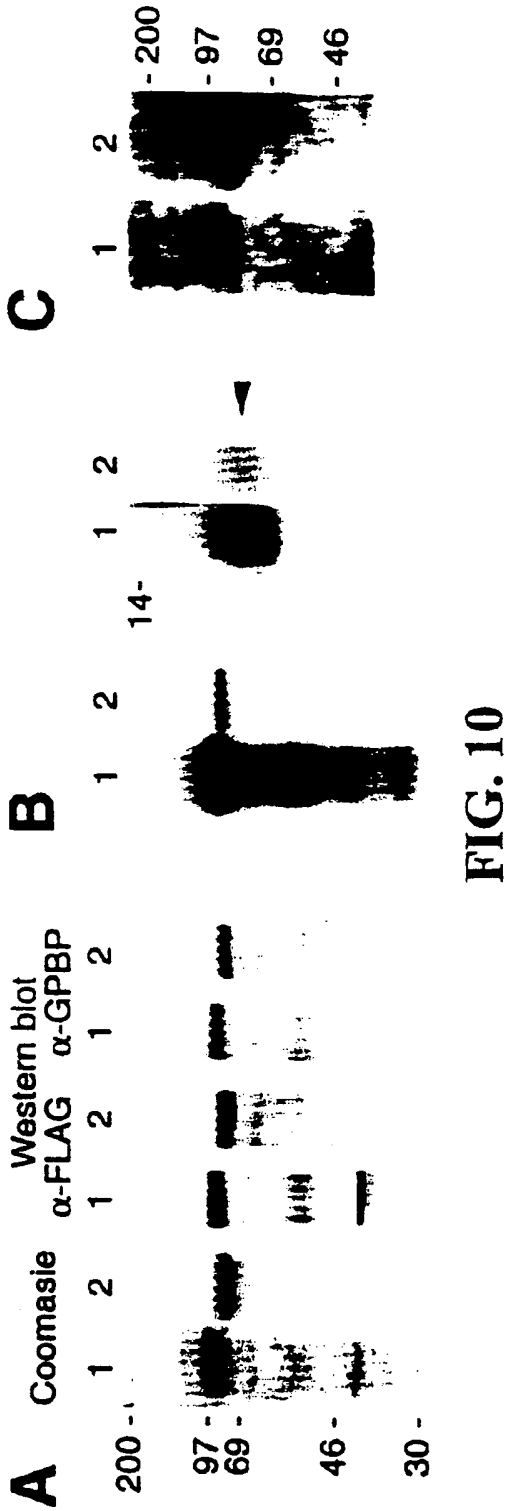

A
Coomasie
1 2
Western blot
α-FLAG
1 2
α-GPBP
1 2
200 -
97 -
69 -
46 -
30 -
B
1 2
14-
C
1 2
- 200
- 97
- 69
- 46

FIG. 10

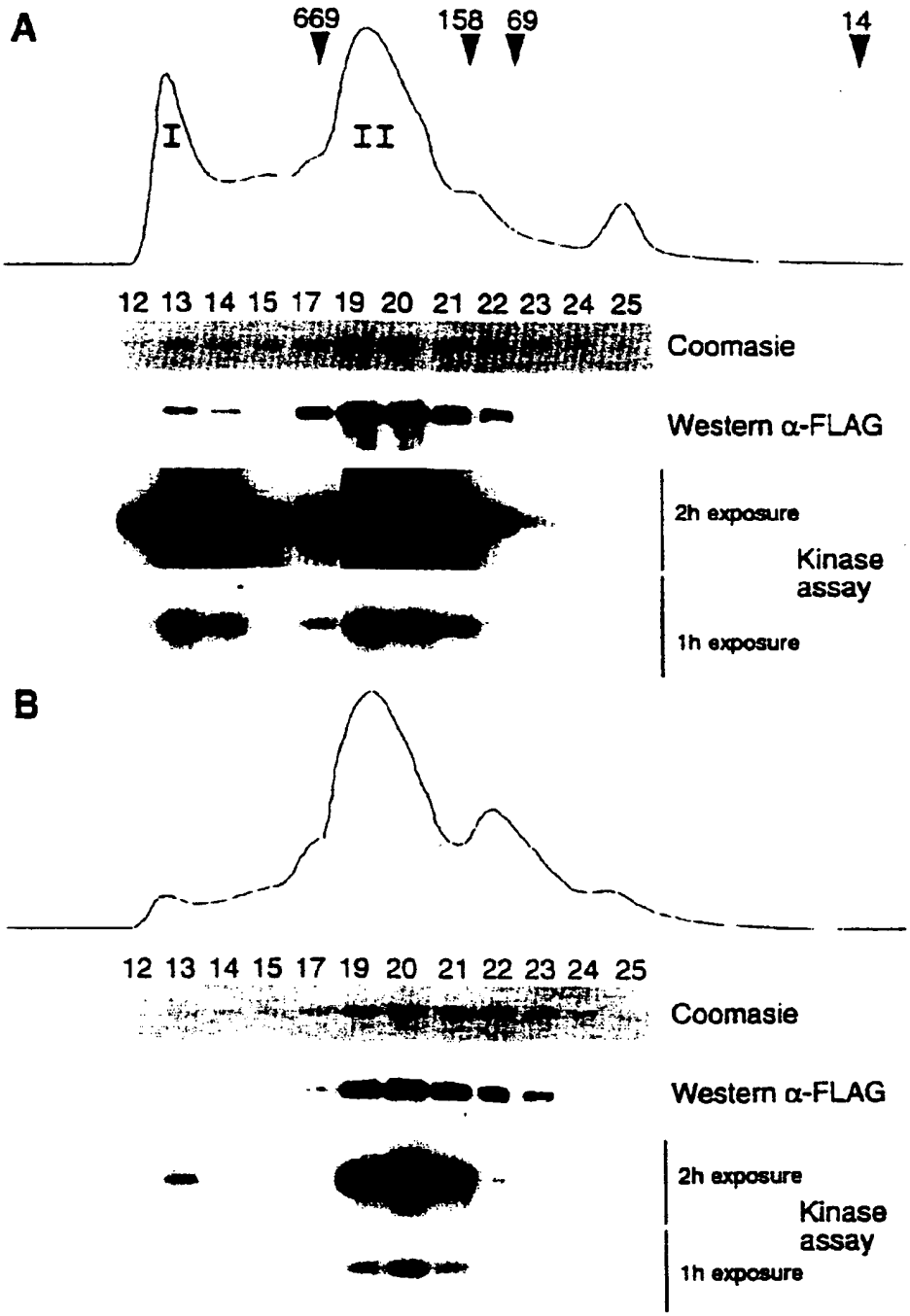

A
669
158
69
14
I
II
12 13 14 15 17 19 20 21 22 23 24 25
Coomasie
Western α-FLAG
2h exposure
Kinase assay
1h exposure
B
12 13 14 15 17 19 20 21 22 23 24 25
Coomasie
Western α-FLAG
2h exposure
Kinase assay
1h exposure

FIG. 11

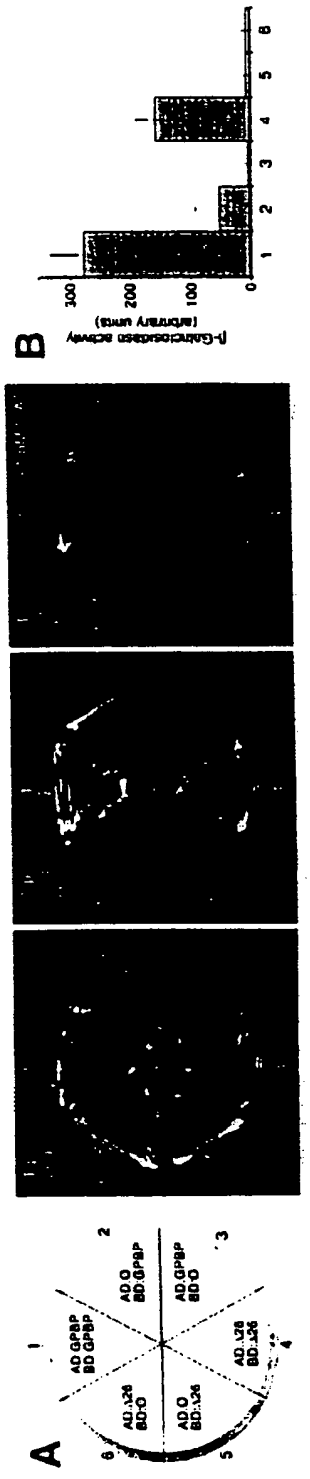

B
β-Galactosidase activity
(arbitrary units)
300
200
100
0
1
2
3
4
5
6
A
AD:0
BD:GPBP
AD:GPBP
BD:0
AD:GPBP
BD:GPBP
AD:0
BD:0
AD:0
BD:126
AD:126
BD:0
AD:126
BD:126

FIG. 12

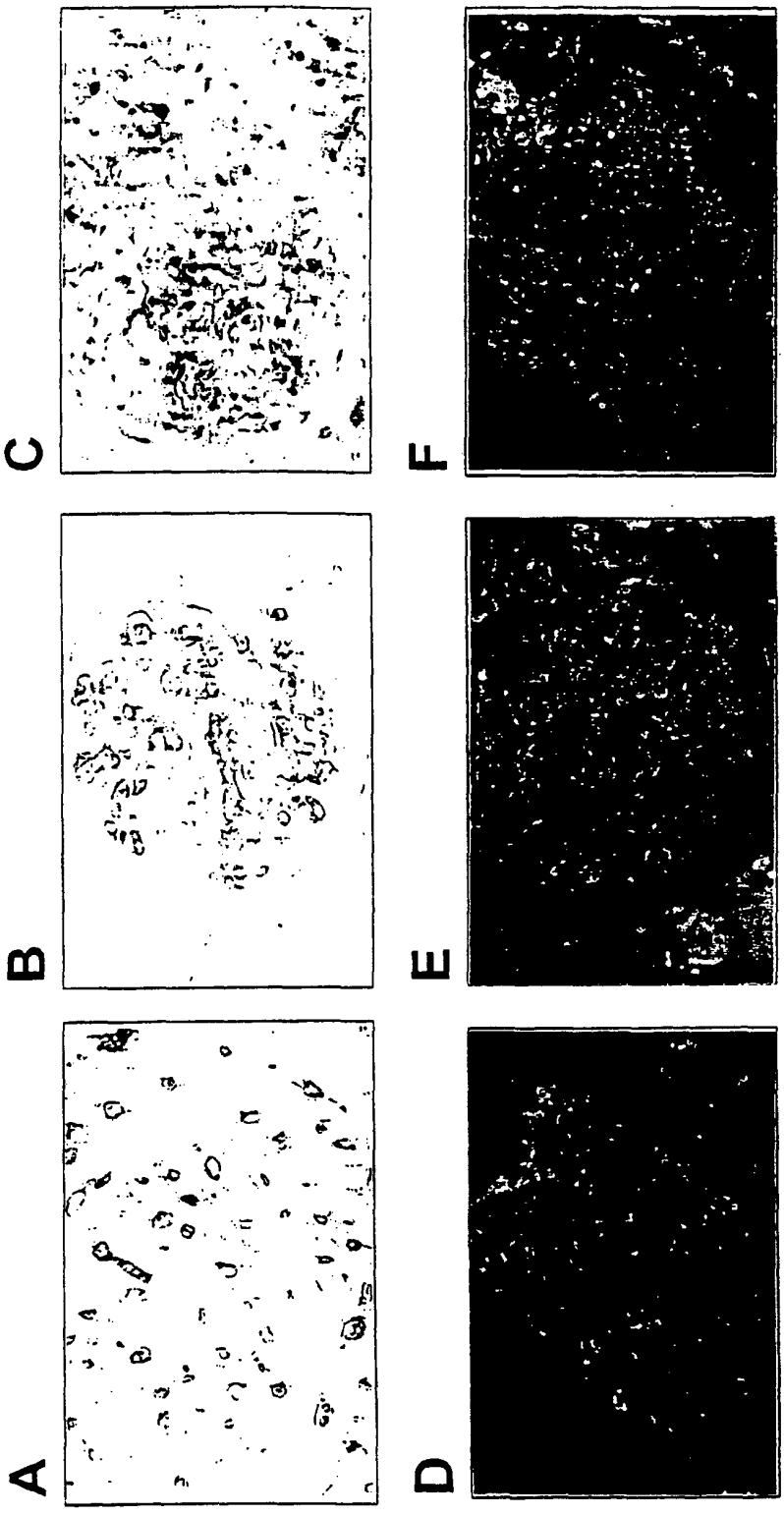


FIG. 13

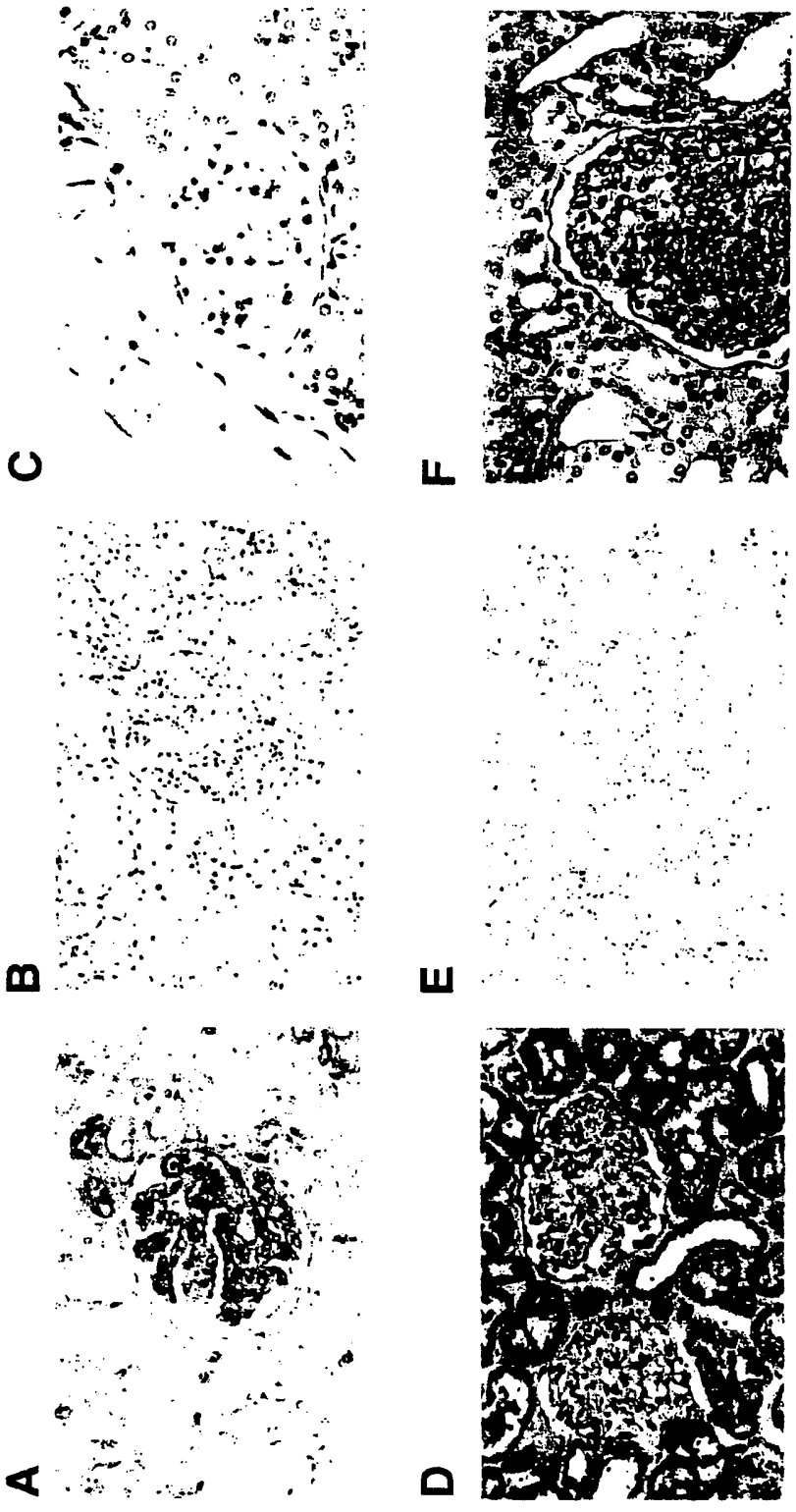


FIG. 14

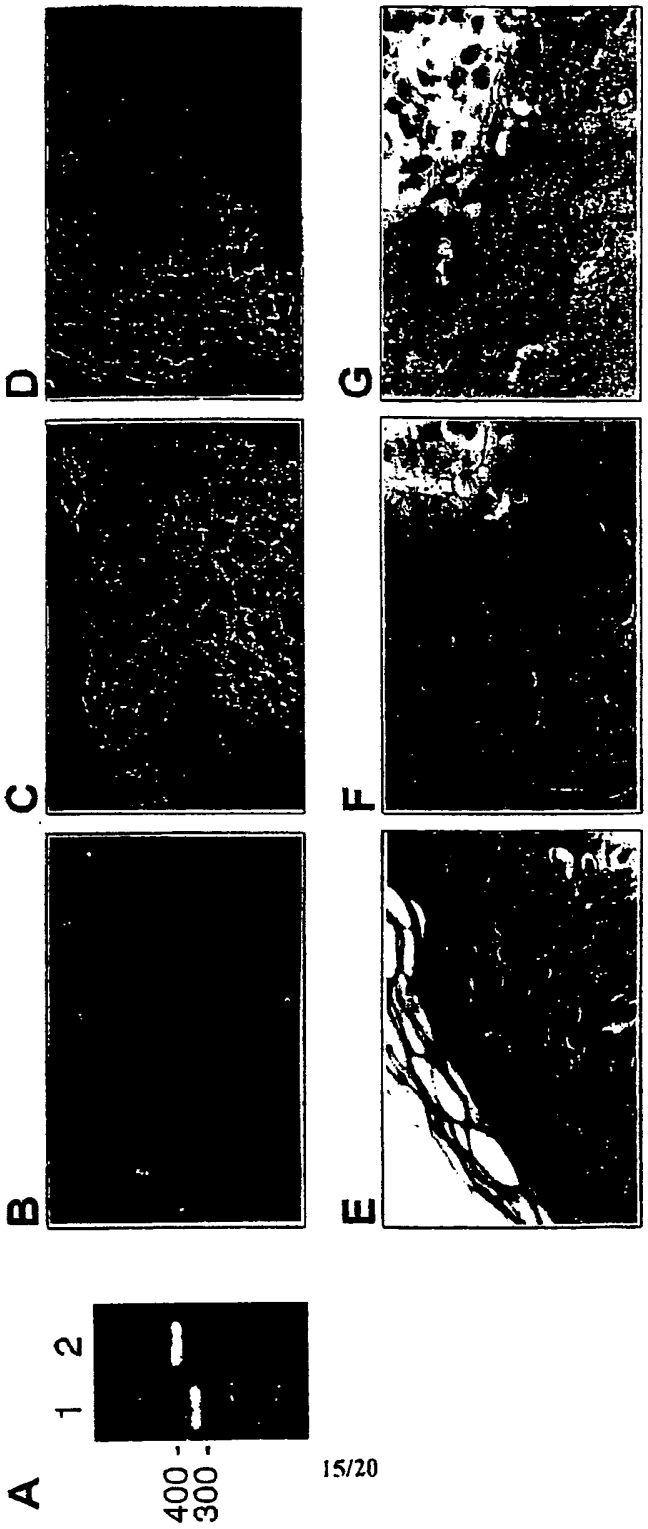
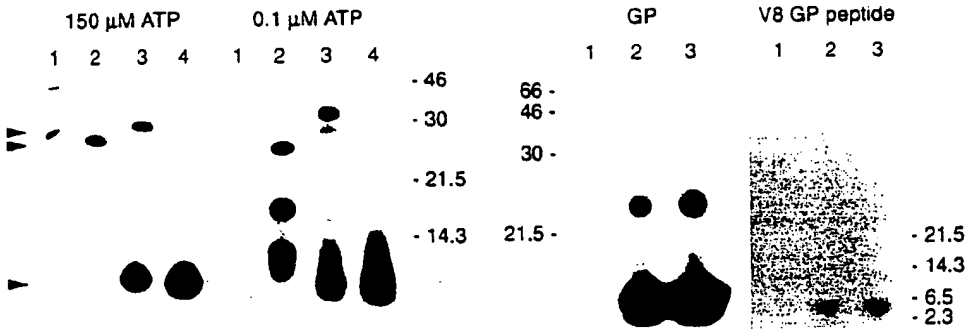


FIG. 15

15/20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0.1 µM ATP
GP
V8 GP peptide
1 2 3 4
1 2 3 4
1 2 3
1 2 3
- 46
- 30
- 21.5
- 14.3
66 -
46 -
30 -
21.5 -
- 21.5
- 14.3
- 6.5
- 2.3

FIG. 16

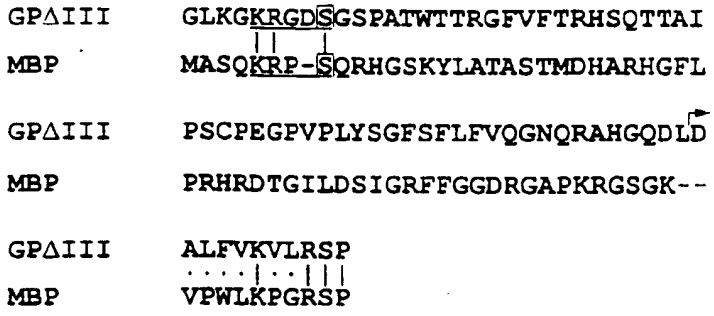
GPΔIII GLKGKRGDSGSPATWTTRGFVFTRHSQTTAI
MBP MASQKRP-SQRHGSKYLATASTMDHARHGFL
GPΔIII PSCPEGPVPLYSGFSFLFVQGNQRAHGQDLD
MBP PRHRDTGILDSIGRFFGGDRGAPKRGSGK--
GPΔIII ALFVKVLRSP
MBP VPWLKPGRSP

FIG. 17

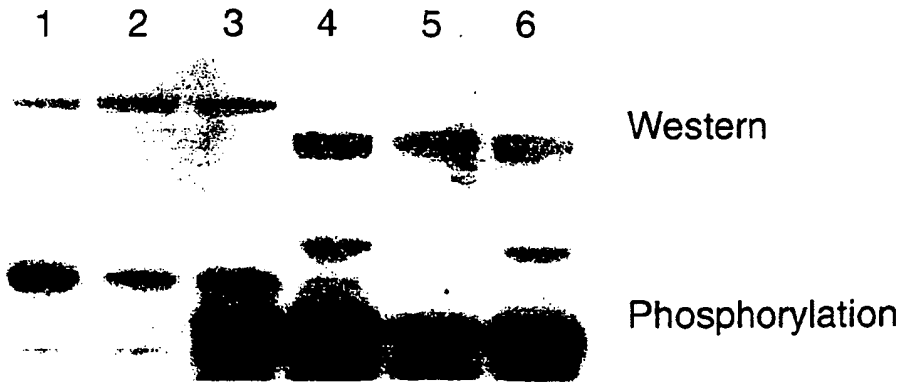

FIG. 18

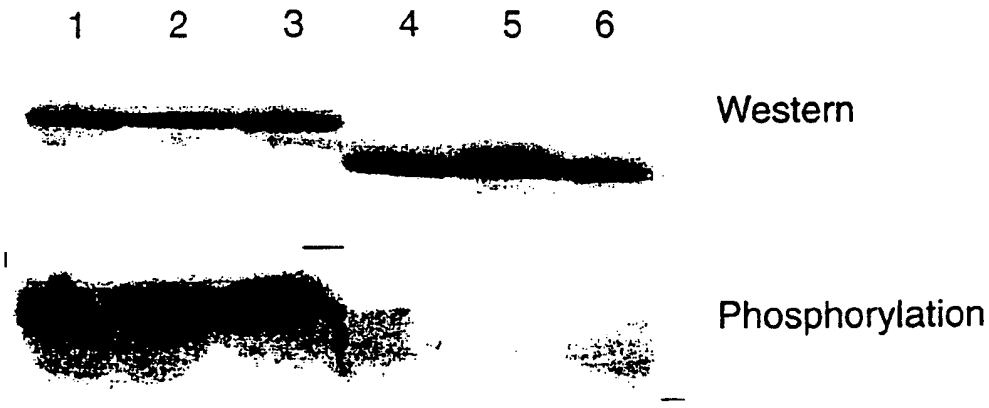

FIG. 19

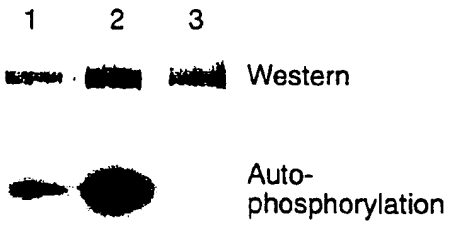

FIG. 20

SEQUENCE LISTING

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<120> Goodpasture Binding Protein

<130> 98-723-B

<140> To Be Assigned
<141> Filed Herewith

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<170> PatentIn Ver. 2.0

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<213> Homo sapiens

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tcgggtggca gcgccgggcg caacgcaggg gtcacggcga cggcggcggc ggctgacggc 180

tggaagggta ggcttcattc accgctcgtc ctccttcctc gctccgctcg gtgtcaggcg 240

cggcggcggc gcggcgggcg gacttcgtcc ctcctcctgc tccccccccac accggagcgg 300

gcactcttcg cttcgccatc ccccgaccct tcaccccgag gactgggcgc ctcctccggc 360

gcagctgagg gagcgggggc cggtctcctg ctcggttgtc gagcctcc atg tcg gat 417
Met Ser Asp
1

aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag acg gag 465
Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
5 10 15

tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg aca aac 513
Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
20 25 30 35

tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat aat gct 561
Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
40 45 50

ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc aga gga 609
Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
55 60 65

tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt gat gaa 657
Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
70 75 80

```
tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt cgt gct   705
Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
     85                  90                  95

cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa cag cac   753
Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
100                 105                 110                 115

aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga cat ggc   801
Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
                120                 125                 130

tca atg gtg tcc ctg gtg tct gga gca agt ggc tac tct gca aca tcc   849
Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser
            135                 140                 145

acc tct tca ttc aag aaa ggc cac agt tta cgt gag aag ttg gct gaa   897
Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu
        150                 155                 160

atg gaa aca ttt aga gac atc tta tgt aga caa gtt gac acg cta cag   945
Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln
    165                 170                 175

aag tac ttt gat gcc tgt gct gat gct gtc tct aag gat gaa ctt caa   993
Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln
180                 185                 190                 195

agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct aca acg cgt   1041
Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg
                200                 205                 210

tct gat ggt gac ttc ttg cat agt acc aac ggc aat aaa gaa aag tta   1089
Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu
            215                 220                 225

ttt cca cat gtg aca cca aaa gga att aat ggt ata gac ttt aaa ggg   1137
Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly
        230                 235                 240

gaa gcg ata act ttt aaa gca act act gct gga atc ctt gca aca ctt   1185
Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu
    245                 250                 255

tct cat tgt att gaa cta atg gtt aaa cgt gag gac agc tgg cag aag   1233
Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys
260                 265                 270                 275

aga ctg gat aag gaa act gag aag aaa aga aga aca gag gaa gca tat   1281
Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr
                280                 285                 290

aaa aat gca atg aca gaa ctt aag aaa aaa tcc cac ttt gga gga cca   1329
Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe Gly Gly Pro
            295                 300                 305

gat tat gaa gaa ggc cct aac agt ctg att aat gaa gaa gag ttc ttt   1377
Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe
        310                 315                 320

gat gct gtt gaa gct gct ctt gac aga caa gat aaa ata gaa gaa cag   1425
```

```
Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln
    325                 330                 335

tca cag agt gaa aag gtg aga tta cat tgg cct aca tcc ttg ccc tct   1473
Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser
340                 345                 350                 355

gga gat gcc ttt tct tct gtg ggg aca cat aga ttt gtc caa aag ccc   1521
Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val Gln Lys Pro
                360                 365                 370

tat agt cgc tct tcc tcc atg tct tcc att gat cta gtc agt gcc tct   1569
Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser
            375                 380                 385

gat gat gtt cac aga ttc agc tcc cag gtt gaa gag atg gtg cag aac   1617
Asp Asp Val His Arg Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn
        390                 395                 400

cac atg act tac tca tta cag gat gta ggc gga gat gcc aat tgg cag   1665
His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln
    405                 410                 415

ttg gtt gta gaa gaa gga gaa atg aag gta tac aga aga gaa gta gaa   1713
Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu
420                 425                 430                 435

gaa aat ggg att gtt ctg gat cct tta aaa gct acc cat gca gtt aaa   1761
Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys
                440                 445                 450

ggc gtc aca gga cat gaa gtc tgc aat tat ttc tgg aat gtt gac gtt   1809
Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val
            455                 460                 465

cgc aat gac tgg gaa aca act ata gaa aac ttt cat gtg gtg gaa aca   1857
Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr
        470                 475                 480

tta gct gat aat gca atc atc att tat caa aca cac aag agg gtg tgg   1905
Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp
    485                 490                 495

cct gct tct cag cga gac gta tta tat ctt tct gtc att cga aag ata   1953
Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile
500                 505                 510                 515

cca gcc ttg act gaa aat gac cct gaa act tgg ata gtt tgt aat ttt   2001
Pro Ala Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe
                520                 525                 530

tct gtg gat cat gac agt gct cct cta aac aac cga tgt gtc cgt gcc   2049
Ser Val Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala
            535                 540                 545

aaa ata aat gtt gct atg att tgt caa acc ttg gta agc cca cca gag   2097
Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu
        550                 555                 560

gga aac cag gaa att agc agg gac aac att cta tgc aag att aca tat   2145
Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr
```

```
    565                 570                 575

gta gct aat gtg aac cct gga gga tgg gca cca gcc tca gtg tta agg   2193
Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg
580                 585                 590                 595

gca gtg gca aag cga gag tat cct aaa ttt cta aaa cgt ttt act tct   2241
Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser
                600                 605                 610

tac gtc caa gaa aaa act gca gga aag cct att ttg ttc tagtattaac    2290
Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
            615                 620

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actaaaattt agttgttgaa agtatttact atgttttt                        2389


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  1               5                  10                  15

Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys
             20                  25                  30

Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
         35                  40                  45

Asn Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
     50                  55                  60

Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80

Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
                 85                  90                  95

Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
            100                 105                 110

Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

Thr Leu Gln Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190
```

```
Glu Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

Thr Thr Arg Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys
    210                 215                 220

Glu Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240

Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser
            260                 265                 270

Trp Gln Lys Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu
        275                 280                 285

Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe
    290                 295                 300

Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser
            340                 345                 350

Leu Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val
    370                 375                 380

Ser Ala Ser Asp Asp Val His Arg Phe Ser Ser Gln Val Glu Glu Met
385                 390                 395                 400

Val Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala
                405                 410                 415

Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg
            420                 425                 430

Glu Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His
        435                 440                 445

Ala Val Lys Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn
    450                 455                 460

Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val
465                 470                 475                 480

Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys
                485                 490                 495

Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Val Ile
            500                 505                 510

Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val
```

```
        515                 520                 525

Cys Asn Phe Ser Val Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys
    530                 535                 540

Val Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser
545                 550                 555                 560

Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys
                565                 570                 575

Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser
            580                 585                 590

Val Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg
        595                 600                 605

Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
    610                 615                 620
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tccctccctg actgaggttg gcatctaggg ggccgagttc aggtggcggc gccgggcgca 180

gcgcaggggt cacggccacg gcggctgacg gctggaaggg caggctttct tcgccgctcg 240

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cgtccctctt cctgttccct cactccccgg agcgggctct cttggcggtg ccatcccccg 360

acccttcacc ccagggacta ggcgcctgca ctggcgcagc tcgcggagcg ggggccggtc 420

tcctgctcgg ctgtcgcgtc tcc atg tcg gat aac cag agc tgg aac tcg tcg 473
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                  15                  20                  25

tgc ggg gtc ctc agc aag tgg aca aac tat att cat gga tgg cag gat   569
Cys Gly Val Leu Ser Lys Trp Thr Asn Tyr Ile His Gly Trp Gln Asp
              30                  35                  40

cgt tgg gta gtt ttg aaa aat aat act ttg agt tac tac aaa tct gaa   617
Arg Trp Val Val Leu Lys Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu
          45                  50                  55
```

```
gat gaa aca gaa tat ggc tgt agg gga tcc atc tgt ctt agc aag gct   665
Asp Glu Thr Glu Tyr Gly Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala
     60                  65                  70

gtg atc acg cct cac gat ttt gat gaa tgc cgg ttt gat atc agt gta   713
Val Ile Thr Pro His Asp Phe Asp Glu Cys Arg Phe Asp Ile Ser Val
 75                  80                  85                  90

aat gat agt gtt tgg tac ctt cga gct cag gac ccg gag cac aga cag   761
Asn Asp Ser Val Trp Tyr Leu Arg Ala Gln Asp Pro Glu His Arg Gln
                 95                 100                 105

caa tgg gta gac gcc att gaa cag cac aag act gaa tcg gga tat gga   809
Gln Trp Val Asp Ala Ile Glu Gln His Lys Thr Glu Ser Gly Tyr Gly
            110                 115                 120

tct gag tcc agc ttg cgt aga cat ggc tca atg gtg tca ctg gtg tct   857
Ser Glu Ser Ser Leu Arg Arg His Gly Ser Met Val Ser Leu Val Ser
        125                 130                 135

gga gcg agt ggc tat tct gct acg tcc acc tct tct ttc aag aaa ggc   905
Gly Ala Ser Gly Tyr Ser Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly
    140                 145                 150

cac agt tta cgt gag aaa ctg gct gaa atg gag aca ttt cgg gac atc   953
His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg Asp Ile
155                 160                 165                 170

ctg tgc cgg cag gtt gat act ctc cag aag tac ttt gat gtc tgt gct   1001
Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Val Cys Ala
                175                 180                 185

gac gct gtc tcc aag gat gag ctt cag agg gat aaa gtc gta gaa gat   1049
Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val Glu Asp
            190                 195                 200

gat gaa gat gac ttc cct aca act cgt tct gat gga gac ttt ttg cac   1097
Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe Leu His
        205                 210                 215

aat acc aat ggt aat aaa gaa aaa tta ttt cca cat gta aca cca aaa   1145
Asn Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr Pro Lys
    220                 225                 230

gga att aat ggc ata gac ttt aaa ggg gaa gca ata act ttt aaa gca   1193
Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala
235                 240                 245                 250

act act gct gga atc ctt gct aca ctt tct cat tgt att gaa tta atg   1241
Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu Leu Met
                255                 260                 265

gta aaa cgg gaa gag agc tgg caa aaa aga cac gat agg gaa gtg gaa   1289
Val Lys Arg Glu Glu Ser Trp Gln Lys Arg His Asp Arg Glu Val Glu
            270                 275                 280

aag agg aga cga gtg gag gaa gcg tac aag aat gtg atg gaa gaa ctt   1337
Lys Arg Arg Arg Val Glu Glu Ala Tyr Lys Asn Val Met Glu Glu Leu
        285                 290                 295

aag aag aaa ccc cgt ttc gga ggg ccg gat tat gaa gaa ggt cca aac   1385
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```
Lys Lys Lys Pro Arg Phe Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn
    300                 305                 310

agt ctg att aat gag gaa gag ttc ttt gat gct gtt gaa gct gct ctt   1433
Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala Ala Leu
315                 320                 325                 330

gac aga caa gat aaa ata gag gaa cag tca cag agt gaa aag gtc agg   1481
Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys Val Arg
                335                 340                 345

tta cac tgg ccc aca tca ttg cca tct gga gac acc ttt tct tct gtc   1529
Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Thr Phe Ser Ser Val
            350                 355                 360

ggg acg cat aga ttt gta caa aag ccc tat agt cgc tct tcc tcc atg   1577
Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met
        365                 370                 375

tct tcc att gat cta gtc agt gcc tct gac gat gtt cac aga ttc agc   1625
Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val His Arg Phe Ser
    380                 385                 390

tcc cag gtt gaa gaa atg gta cag aac cac atg aac tat tca tta cag   1673
Ser Gln Val Glu Glu Met Val Gln Asn His Met Asn Tyr Ser Leu Gln
395                 400                 405                 410

gat gta ggt ggt gat gca aat tgg caa ctg gtt gtt gaa gaa gga gaa   1721
Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
                415                 420                 425

atg aag gta tac aga aga gaa gtg gaa gaa aat gga att gtt ctg gat   1769
Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
            430                 435                 440

cct ttg aaa gct act cat gca gtt aaa ggt gtt aca gga cat gag gtc   1817
Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
        445                 450                 455

tgc aat tac ttt tgg aat gtt gat gtt cgc aat gac tgg gaa act act   1865
Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
    460                 465                 470

ata gaa aac ttt cat gtg gtg gaa aca tta gct gat aat gca atc atc   1913
Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
475                 480                 485                 490

gtt tat caa acg cac aag aga gta tgg ccc gct tct cag aga gac gta   1961
Val Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
                495                 500                 505

ctg tat ctt tct gct att cga aag atc cca gcc ttg act gaa aat gat   2009
Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp
            510                 515                 520

cct gaa act tgg ata gtt tgt aat ttt tct gtg gat cat gat agt gct   2057
Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala
        525                 530                 535

cct ctg aac aat cga tgt gtc cgt gcc aaa atc aat att gct atg att   2105
Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Ile Ala Met Ile
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    540                 545                 550

tgt caa act tta gta agc cca cca gag gga gac cag gag ata agc aga   2153
Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asp Gln Glu Ile Ser Arg
555                 560                 565                 570

gac aac att ctg tgc aag atc acg tat gta gct aat gtg aac cca gga   2201
Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
                575                 580                 585

gga tgg gcg cca gct tcg gtc tta aga gca gtg gca aag cga gaa tac   2249
Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
            590                 595                 600

cct aag ttt cta aaa cgt ttt act tct tat gtc caa gaa aaa act gca   2297
Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala
        605                 610                 615

gga aaa cca att ttg ttt tagtattaac agtgactgaa gcaaggctgc          2345
Gly Lys Pro Ile Leu Phe
    620

gtgacgttcc atgttggaga aaggagggaa aaaataaaaa gaatcctcta agctggaacg 2405

taggatctac agccttgtct gtggcccaag aagaaacatt gcaatcgtaa agctgggtat 2465

ccagcactag ccatctcctg ctaggcctcc tcgctcagcg tgtaactata aatacatgta 2525

gaatcacatg gatatggcta tatttttatt tgcttgctcc ttggagtgaa aacaaataac 2585

tttgaattac aactaggaat taaccgatgc tttaattttg aggaactttt tcagaatttt 2645

ttatttacca tggtccaacc taagatcctc agttgtatca agtttttgtg cacaaaagaa 2705

aagcacaaaa gttgaacgca cctgaaggca tgtgctctct gtgcaacaaa tactcag    2762
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<210> 4
<211> 624
<212> PRT
<213> Mus musculus

<400> 4

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Met Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro
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Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys
             20                  25                  30

Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
         35                  40                  45

Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
     50                  55                  60

Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80

Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
                 85                  90                  95
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```
Leu Arg Ala Gln Asp Pro Glu His Arg Gln Gln Trp Val Asp Ala Ile
            100                 105                 110

Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

Thr Leu Gln Lys Tyr Phe Asp Val Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190

Glu Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
    210                 215                 220

Glu Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240

Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Glu Ser
            260                 265                 270

Trp Gln Lys Arg His Asp Arg Glu Val Glu Lys Arg Arg Arg Val Glu
        275                 280                 285

Glu Ala Tyr Lys Asn Val Met Glu Glu Leu Lys Lys Lys Pro Arg Phe
    290                 295                 300

Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser
            340                 345                 350

Leu Pro Ser Gly Asp Thr Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val
    370                 375                 380

Ser Ala Ser Asp Asp Val His Arg Phe Ser Ser Gln Val Glu Glu Met
385                 390                 395                 400

Val Gln Asn His Met Asn Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala
                405                 410                 415

Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg
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            420                 425                 430

Glu Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His
        435                 440                 445

Ala Val Lys Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn
    450                 455                 460

Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val
465                 470                 475                 480

Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Val Tyr Gln Thr His Lys
                485                 490                 495

Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Ala Ile
            500                 505                 510

Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val
        515                 520                 525

Cys Asn Phe Ser Val Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys
    530                 535                 540

Val Arg Ala Lys Ile Asn Ile Ala Met Ile Cys Gln Thr Leu Val Ser
545                 550                 555                 560

Pro Pro Glu Gly Asp Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys
                565                 570                 575

Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser
            580                 585                 590

Val Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg
        595                 600                 605

Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
    610                 615                 620
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<210> 5
<211> 2361
<212> DNA
<213> Bos taurus

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<222> (421)..(2292)

<400> 5
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ttccctcttc ccgtctttct cctcttttcc tctcccccga ggttggcatc gaggggggca 120

aattcgggcg gcggcgccgg gcgcagcgca ggggtcacaa cgacggcgac ggctgacggt 180

tggaagggca ggcttccttc gcccctcgac ctccttcccc ggtccgcttg gtgtcaggcg 240

cggcggcggc ggcggcggcg gcgcggcggg cggactccat ccctcctccc gctccctcct 300

gcaccggagc gggcactcct tccttcgcca tcccccgacc cttcaccccg gggactgggc 360

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gcctccaccg gcgcagctca gggagcgggg gccggtctcc tgctcggctg tcgcgcctcc 420

atg tcg gat aac cag agc tgg aac tcg tcg ggc tcg gag gag gat ccg   468
Met Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro
  1               5                  10                  15

gag acg gag tcc ggg ccg ccg gtg gag cgc tgc gga gtc ctc aac aag   516
Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Asn Lys
            20                  25                  30

tgg aca aac tat att cat ggg tgg cag gat cgc tgg gta gtt ttg aaa   564
Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
         35                  40                  45

aat aac act ctg agt tac tac aaa tct gaa gat gag aca gag tat ggc   612
Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
     50                  55                  60

tgc aga gga tcc atc tgt ctt agc aag gct gtc atc acg cct cat gat   660
Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80

ttt gat gaa tgc cga ttt gat att agt gta aat gat agt gtt tgg tat   708
Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
                 85                  90                  95

ctt cgt gct caa gat cca gat cac aga cag cag tgg ata gat gcc att   756
Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
            100                 105                 110

gaa cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt   804
Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

cga cat ggc tcc atg gta tca ttg gta tcc gga gca agt ggc tat tct   852
Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

gca aca tcc acc tcc tca ttc aag aag ggc cac agt tta cgt gag aaa   900
Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

ctg gct gaa atg gaa acc ttt aga gat ata ctg tgt aga caa gtt gat   948
Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

acc cta cag aag ttc ttt gat gcc tgt gct gat gct gtc tcc aag gat   996
Thr Leu Gln Lys Phe Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190

gaa ttt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct   1044
Glu Phe Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

acg aca cgt tct gat gga gac ttc ttg cat aat acc aat ggc aat aag   1092
Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
    210                 215                 220

gaa aag gta ttt cca cat gta aca cca aaa gga att aat ggt ata gac   1140
Glu Lys Val Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240
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```
ttt aaa ggt gag gcg ata act ttt aaa gca act act gcc gga atc ctt   1188
Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

gct aca ctt tct cat tgt att gag ctg atg gta aaa cgt gag gac agc   1236
Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser
            260                 265                 270

tgg caa aag aga atg gac aag gaa act gag aag aga aga aga gtg gag   1284
Trp Gln Lys Arg Met Asp Lys Glu Thr Glu Lys Arg Arg Arg Val Glu
        275                 280                 285

gaa gca tac aaa aat gcc atg aca gaa ctt aag aaa aaa tcc cac ttt   1332
Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe
    290                 295                 300

gga gga cca gat tat gag gaa ggc cca aac agt ttg att aat gaa gag   1380
Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

gag ttc ttt gat gct gtt gaa gct gct ctt gac aga caa gat aaa ata   1428
Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

gaa gaa cag tcg cag agt gaa aag gtc agg tta cat tgg tct act tca   1476
Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Ser Thr Ser
            340                 345                 350

atg cca tct gga gat gcc ttt tct tct gtg ggg act cat aga ttt gtc   1524
Met Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

caa aag ccc tat agt cgc tct tcc tcc atg tct tcc att gat cta gtc   1572
Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val
    370                 375                 380

agt gcc tct gac ggt gtt cac aga ttc agc tcc cag gtt gaa gag atg   1620
Ser Ala Ser Asp Gly Val His Arg Phe Ser Ser Gln Val Glu Glu Met
385                 390                 395                 400

gtg cag aac cac atg acc tat tca ttg cag gat gta ggt ggg gac gcc   1668
Val Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala
                405                 410                 415

aac tgg cag ttg gtt gta gaa gaa ggg gag atg aag gta tat aga aga   1716
Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg
            420                 425                 430

gaa gta gaa gaa aat ggg att gtt ctg gat cct ttg aaa gct acc cat   1764
Glu Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His
        435                 440                 445

gca gtt aaa ggc gtt aca gga cac gag gtc tgc aat tac ttc tgg aat   1812
Ala Val Lys Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn
    450                 455                 460

gtt gat gtt cgc aat gat tgg gaa aca act ata gaa aac ttt cat gtg   1860
Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val
465                 470                 475                 480
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gtg gaa aca tta gct gat aat gca atc atc att tat caa acg cac aag   1908
Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys
                485                 490                 495

aga gtg tgg cca gcc tct cag cgg gat gtc tta tat ctg tct gcc att   1956
Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Ala Ile
            500                 505                 510

cga aag ata cca gct ttg aat gaa aat gac ccg gag act tgg ata gtt   2004
Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp Pro Glu Thr Trp Ile Val
        515                 520                 525

tgt aat ttt tct gta gat cac agc agt gct cct cta aac aat cga tgt   2052
Cys Asn Phe Ser Val Asp His Ser Ser Ala Pro Leu Asn Asn Arg Cys
    530                 535                 540

gtc cgt gcc aaa ata aac gtt gct atg att tgt cag acc ttg gtg agc   2100
Val Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser
545                 550                 555                 560

ccc cca gag gga aac cag gag att agc agg gac aac att cta tgc aag   2148
Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys
                565                 570                 575

att aca tac gtg gcc aat gta aac cct gga gga tgg gcc cca gcc tca   2196
Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser
            580                 585                 590

gtg tta cgg gca gtg gca aag cga gaa tat cca aag ttt cta aag cgt   2244
Val Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg
        595                 600                 605

ttt act tct tac gta caa gaa aaa act gca gga aaa cct att ttg ttc   2292
Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
    610                 615                 620

tagtattaac agtgactgaa gcaaggctgt gtgacattcc atgttggagg aaaaaaaaaa 2352

aaaaaaaaa                                                        2361
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<210> 6
<211> 624
<212> PRT
<213> Bos taurus

<400> 6

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Met Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro
  1               5                  10                  15

Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Asn Lys
             20                  25                  30

Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
         35                  40                  45

Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
     50                  55                  60

Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80
```

```
Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
                 85                  90                  95

Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
            100                 105                 110

Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

Thr Leu Gln Lys Phe Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190

Glu Phe Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
    210                 215                 220

Glu Lys Val Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240

Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser
            260                 265                 270

Trp Gln Lys Arg Met Asp Lys Glu Thr Glu Lys Arg Arg Arg Val Glu
        275                 280                 285

Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe
    290                 295                 300

Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Ser Thr Ser
            340                 345                 350

Met Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val
    370                 375                 380

Ser Ala Ser Asp Gly Val His Arg Phe Ser Ser Gln Val Glu Glu Met
385                 390                 395                 400
```

```
Val Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala
                405                 410                 415

Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg
            420                 425                 430

Glu Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His
        435                 440                 445

Ala Val Lys Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn
    450                 455                 460

Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val
465                 470                 475                 480

Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys
                485                 490                 495

Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Ala Ile
            500                 505                 510

Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp Pro Glu Thr Trp Ile Val
        515                 520                 525

Cys Asn Phe Ser Val Asp His Ser Ser Ala Pro Leu Asn Asn Arg Cys
    530                 535                 540

Val Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser
545                 550                 555                 560

Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys
                565                 570                 575

Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser
            580                 585                 590

Val Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg
        595                 600                 605

Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
    610                 615                 620
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<210> 7
<211> 2187
<212> DNA
<213> Homo sapiens

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<222> (391)..(2187)

<400> 7
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cttttccttc cctatttgaa attggcatcg agggggctaa gttcgggtgg cagcgccggg 120

cgcaacgcag gggtcacggc gacggcggcg gcggctgacg gctggaaggg taggcttcat 180

tcaccgctcg tcctccttcc tcgctccgct cggtgtcagg cgcggcggcg gcgcggcggg 240

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cggacttcgt ccctcctcct gctccccccc acaccggagc gggcactctt cgcttcgcca 300

tcccccgacc cttcaccccg aggactgggc gcctcctccg gcgcagctga gggagcgggg 360

gccggtctcc tgctcggttg tcgagcctcc atg tcg gat aat cag agc tgg aac  414
                                Met Ser Asp Asn Gln Ser Trp Asn
                                  1               5

tcg tcg ggc tcg gag gag gat cca gag acg gag tct ggg ccg cct gtg   462
Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu Ser Gly Pro Pro Val
     10                  15                  20

gag cgc tgc ggg gtc ctc agt aag tgg aca aac tac att cat ggg tgg   510
Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn Tyr Ile His Gly Trp
 25                  30                  35                  40

cag gat cgt tgg gta gtt ttg aaa aat aat gct ctg agt tac tac aaa   558
Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala Leu Ser Tyr Tyr Lys
                 45                  50                  55

tct gaa gat gaa aca gag tat ggc tgc aga gga tcc atc tgt ctt agc   606
Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly Ser Ile Cys Leu Ser
             60                  65                  70

aag gct gtc atc aca cct cac gat ttt gat gaa tgt cga ttt gat att   654
Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu Cys Arg Phe Asp Ile
         75                  80                  85

agt gta aat gat agt gtt tgg tat ctt cgt gct cag gat cca gat cat   702
Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala Gln Asp Pro Asp His
     90                  95                 100

aga cag caa tgg ata gat gcc att gaa cag cac aag act gaa tct gga   750
Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His Lys Thr Glu Ser Gly
105                 110                 115                 120

tat gga tct gaa tcc agc ttg cgt cga cat ggc tca atg gtg tcc ctg   798
Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly Ser Met Val Ser Leu
                125                 130                 135

gtg tct gga gca agt ggc tac tct gca aca tcc acc tct tca ttc aag   846
Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser Thr Ser Ser Phe Lys
            140                 145                 150

aaa ggc cac agt tta cgt gag aag ttg gct gaa atg gaa aca ttt aga   894
Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg
        155                 160                 165

gac atc tta tgt aga caa gtt gac acg cta cag aag tac ttt gat gcc   942
Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Ala
    170                 175                 180

tgt gct gat gct gtc tct aag gat gaa ctt caa agg gat aaa gtg gta   990
Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val
185                 190                 195                 200

gaa gat gat gaa gat gac ttt cct aca acg cgt tct gat ggt gac ttc  1038
Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe
                205                 210                 215

ttg cat agt acc aac ggc aat aaa gaa aag tta ttt cca cat gtg aca  1086
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Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr
            220                 225                 230

cca aaa gga att aat ggt ata gac ttt aaa ggg gaa gcg ata act ttt   1134
Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe
        235                 240                 245

aaa gca act act gct gga atc ctt gca aca ctt tct cat tgt att gaa   1182
Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu
    250                 255                 260

cta atg gtt aaa cgt gag gac agc tgg cag aag aga ctg gat aag gaa   1230
Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp Lys Glu
265                 270                 275                 280

act gag aag aaa aga aga aca gag gaa gca tat aaa aat gca atg aca   1278
Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr Lys Asn Ala Met Thr
                285                 290                 295

gaa ctt aag aaa aaa tcc cac ttt gga gga cca gat tat gaa gaa ggc   1326
Glu Leu Lys Lys Lys Ser His Phe Gly Gly Pro Asp Tyr Glu Glu Gly
            300                 305                 310

cct aac agt ctg att aat gaa gaa gag ttc ttt gat gct gtt gaa gct   1374
Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala
        315                 320                 325

gct ctt gac aga caa gat aaa ata gaa gaa cag tca cag agt gaa aag   1422
Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys
    330                 335                 340

gtg aga tta cat tgg cct aca tcc ttg ccc tct gga gat gcc ttt tct   1470
Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala Phe Ser
345                 350                 355                 360

tct gtg ggg aca cat aga ttt gtc caa aag gtt gaa gag atg gtg cag   1518
Ser Val Gly Thr His Arg Phe Val Gln Lys Val Glu Glu Met Val Gln
                365                 370                 375

aac cac atg act tac tca tta cag gat gta ggc gga gat gcc aat tgg   1566
Asn His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp
            380                 385                 390

cag ttg gtt gta gaa gaa gga gaa atg aag gta tac aga aga gaa gta   1614
Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val
        395                 400                 405

gaa gaa aat ggg att gtt ctg gat cct tta aaa gct acc cat gca gtt   1662
Glu Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val
    410                 415                 420

aaa ggc gtc aca gga cat gaa gtc tgc aat tat ttc tgg aat gtt gac   1710
Lys Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp
425                 430                 435                 440

gtt cgc aat gac tgg gaa aca act ata gaa aac ttt cat gtg gtg gaa   1758
Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu
                445                 450                 455

aca tta gct gat aat gca atc atc att tat caa aca cac aag agg gtg   1806
Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val
```

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            460                 465                 470

tgg cct gct tct cag cga gac gta tta tat ctt tct gtc att cga aag   1854
Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys
        475                 480                 485

ata cca gcc ttg act gaa aat gac cct gaa act tgg ata gtt tgt aat   1902
Ile Pro Ala Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn
    490                 495                 500

ttt tct gtg gat cat gac agt gct cct cta aac aac cga tgt gtc cgt   1950
Phe Ser Val Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg
505                 510                 515                 520

gcc aaa ata aat gtt gct atg att tgt caa acc ttg gta agc cca cca   1998
Ala Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro
                525                 530                 535

gag gga aac cag gaa att agc agg gac aac att cta tgc aag att aca   2046
Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr
            540                 545                 550

tat gta gct aat gtg aac cct gga gga tgg gca cca gcc tca gtg tta   2094
Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu
        555                 560                 565

agg gca gtg gca aag cga gag tat cct aaa ttt cta aaa cgt ttt act   2142
Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr
    570                 575                 580

tct tac gtc caa gaa aaa act gca gga aag cct att ttg ttc tag       2187
Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
585                 590                 595
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Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys
             20                  25                  30

Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
         35                  40                  45

Asn Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
     50                  55                  60

Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80

Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
                 85                  90                  95

Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
            100                 105                 110
```

```
Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

Thr Leu Gln Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190

Glu Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

Thr Thr Arg Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys
    210                 215                 220

Glu Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240

Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser
            260                 265                 270

Trp Gln Lys Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu
        275                 280                 285

Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe
    290                 295                 300

Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser
            340                 345                 350

Leu Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

Gln Lys Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln
    370                 375                 380

Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
385                 390                 395                 400

Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
                405                 410                 415

Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
            420                 425                 430
```

```
Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
        435                 440                 445

Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
    450                 455                 460

Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
465                 470                 475                 480

Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp
                485                 490                 495

Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala
            500                 505                 510

Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile
        515                 520                 525

Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg
    530                 535                 540

Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
545                 550                 555                 560

Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
                565                 570                 575

Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala
            580                 585                 590

Gly Lys Pro Ile Leu Phe
        595
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tccctccctg actgaggttg gcatctaggg ggccgagttc aggtggcggc gccgggcgca 180

gcgcaggggt cacggccacg gcggctgacg gctggaaggg caggctttct tcgccgctcg 240

tcctccttcc ccggtccgct cggtgtcagg cgcggcggcg gcggcgcggc gggcgcgctt 300

cgtccctctt cctgttccct cactccccgg agcgggctct cttggcggtg ccatcccccg 360

acccttcacc ccagggacta ggcgcctgca ctggcgcagc tcgcggagcg ggggccggtc 420

tcctgctcgg ctgtcgcgtc tcc atg tcg gat aac cag agc tgg aac tcg tcg 473
                        Met Ser Asp Asn Gln Ser Trp Asn Ser Ser
                          1   5   10

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ggc tcg gag gag gat ccg gag acg gag tcc ggg ccg cct gtg gag cgc   521
Gly Ser Glu Glu Asp Pro Glu Thr Glu Ser Gly Pro Pro Val Glu Arg
                15                  20                  25

tgc ggg gtc ctc agc aag tgg aca aac tat att cat gga tgg cag gat   569
Cys Gly Val Leu Ser Lys Trp Thr Asn Tyr Ile His Gly Trp Gln Asp
            30                  35                  40

cgt tgg gta gtt ttg aaa aat aat act ttg agt tac tac aaa tct gaa   617
Arg Trp Val Val Leu Lys Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu
        45                  50                  55

gat gaa aca gaa tat ggc tgt agg gga tcc atc tgt ctt agc aag gct   665
Asp Glu Thr Glu Tyr Gly Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala
    60                  65                  70

gtg atc acg cct cac gat ttt gat gaa tgc cgg ttt gat atc agt gta   713
Val Ile Thr Pro His Asp Phe Asp Glu Cys Arg Phe Asp Ile Ser Val
75                  80                  85                  90

aat gat agt gtt tgg tac ctt cga gct cag gac ccg gag cac aga cag   761
Asn Asp Ser Val Trp Tyr Leu Arg Ala Gln Asp Pro Glu His Arg Gln
                95                  100                 105

caa tgg gta gac gcc att gaa cag cac aag act gaa tcg gga tat gga   809
Gln Trp Val Asp Ala Ile Glu Gln His Lys Thr Glu Ser Gly Tyr Gly
            110                 115                 120

tct gag tcc agc ttg cgt aga cat ggc tca atg gtg tca ctg gtg tct   857
Ser Glu Ser Ser Leu Arg Arg His Gly Ser Met Val Ser Leu Val Ser
        125                 130                 135

gga gcg agt ggc tat tct gct acg tcc acc tct tct ttc aag aaa ggc   905
Gly Ala Ser Gly Tyr Ser Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly
    140                 145                 150

cac agt tta cgt gag aaa ctg gct gaa atg gag aca ttt cgg gac atc   953
His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg Asp Ile
155                 160                 165                 170

ctg tgc cgg cag gtt gat act ctc cag aag tac ttt gat gtc tgt gct   1001
Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Val Cys Ala
                175                 180                 185

gac gct gtc tcc aag gat gag ctt cag agg gat aaa gtc gta gaa gat   1049
Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val Glu Asp
            190                 195                 200

gat gaa gat gac ttc cct aca act cgt tct gat gga gac ttt ttg cac   1097
Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe Leu His
        205                 210                 215

aat acc aat ggt aat aaa gaa aaa tta ttt cca cat gta aca cca aaa   1145
Asn Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr Pro Lys
    220                 225                 230

gga att aat ggc ata gac ttt aaa ggg gaa gca ata act ttt aaa gca   1193
Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala
235                 240                 245                 250
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act act gct gga atc ctt gct aca ctt tct cat tgt att gaa tta atg   1241
Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu Leu Met
                255                 260                 265

gta aaa cgg gaa gag agc tgg caa aaa aga cac gat agg gaa gtg gaa   1289
Val Lys Arg Glu Glu Ser Trp Gln Lys Arg His Asp Arg Glu Val Glu
            270                 275                 280

aag agg aga cga gtg gag gaa gcg tac aag aat gtg atg gaa gaa ctt   1337
Lys Arg Arg Arg Val Glu Glu Ala Tyr Lys Asn Val Met Glu Glu Leu
        285                 290                 295

aag aag aaa ccc cgt ttc gga ggg ccg gat tat gaa gaa ggt cca aac   1385
Lys Lys Lys Pro Arg Phe Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn
    300                 305                 310

agt ctg att aat gag gaa gag ttc ttt gat gct gtt gaa gct gct ctt   1433
Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala Ala Leu
315                 320                 325                 330

gac aga caa gat aaa ata gag gaa cag tca cag agt gaa aag gtc agg   1481
Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys Val Arg
                335                 340                 345

tta cac tgg ccc aca tca ttg cca tct gga gac acc ttt tct tct gtc   1529
Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Thr Phe Ser Ser Val
            350                 355                 360

ggg acg cat aga ttt gta caa aag gtt gaa gaa atg gta cag aac cac   1577
Gly Thr His Arg Phe Val Gln Lys Val Glu Glu Met Val Gln Asn His
        365                 370                 375

atg aac tat tca tta cag gat gta ggt ggt gat gca aat tgg caa ctg   1625
Met Asn Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu
    380                 385                 390

gtt gtt gaa gaa gga gaa atg aag gta tac aga aga gaa gtg gaa gaa   1673
Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu
395                 400                 405                 410

aat gga att gtt ctg gat cct ttg aaa gct act cat gca gtt aaa ggt   1721
Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly
                415                 420                 425

gtt aca gga cat gag gtc tgc aat tac ttt tgg aat gtt gat gtt cgc   1769
Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg
            430                 435                 440

aat gac tgg gaa act act ata gaa aac ttt cat gtg gtg gaa aca tta   1817
Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu
        445                 450                 455

gct gat aat gca atc atc gtt tat caa acg cac aag aga gta tgg ccc   1865
Ala Asp Asn Ala Ile Ile Val Tyr Gln Thr His Lys Arg Val Trp Pro
    460                 465                 470

gct tct cag aga gac gta ctg tat ctt tct gct att cga aag atc cca   1913
Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro
475                 480                 485                 490

gcc ttg act gaa aat gat cct gaa act tgg ata gtt tgt aat ttt tct   1961
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Ala Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser
                495                 500                 505

gtg gat cat gat agt gct cct ctg aac aat cga tgt gtc cgt gcc aaa   2009
Val Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys
            510                 515                 520

atc aat att gct atg att tgt caa act tta gta agc cca cca gag gga   2057
Ile Asn Ile Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly
        525                 530                 535

gac cag gag ata agc aga gac aac att ctg tgc aag atc acg tat gta   2105
Asp Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val
    540                 545                 550

gct aat gtg aac cca gga gga tgg gcg cca gct tcg gtc tta aga gca   2153
Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg Ala
555                 560                 565                 570

gtg gca aag cga gaa tac cct aag ttt cta aaa cgt ttt act tct tat   2201
Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr
                575                 580                 585

gtc caa gaa aaa act gca gga aaa cca att ttg ttt tagtattaac        2247
Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
            590                 595

agtgactgaa gcaaggctgc gtgacgttcc atgttggaga aaggagggaa aaaataaaaa 2307

gaatcctcta agctggaacg taggatctac agccttgtct gtggcccaag aagaaacatt 2367

gcaatcgtaa agctgggtat ccagcactag ccatctcctg ctaggcctcc tcgctcagcg 2427

tgtaactata aatacatgta gaatcacatg gatatggcta tatttttatt tgcttgctcc 2487

ttggagtgaa aacaaataac tttgaattac aactaggaat taaccgatgc tttaattttg 2547

aggaactttt tcagaatttt ttatttacca tggtccaacc taagatcctc agttgtatca 2607

agtttttgtg cacaaaagaa aagcacaaaa gttgaacgca cctgaaggca tgtgctctct 2667

gtgcaacaaa tactcag                                               2684


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            20                  25                  30

Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
        35                  40                  45

Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
    50                  55                  60
```

```
Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80

Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
                 85                  90                  95

Leu Arg Ala Gln Asp Pro Glu His Arg Gln Gln Trp Val Asp Ala Ile
            100                 105                 110

Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

Thr Leu Gln Lys Tyr Phe Asp Val Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190

Glu Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
    210                 215                 220

Glu Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240

Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Glu Ser
            260                 265                 270

Trp Gln Lys Arg His Asp Arg Glu Val Glu Lys Arg Arg Arg Val Glu
        275                 280                 285

Glu Ala Tyr Lys Asn Val Met Glu Glu Leu Lys Lys Lys Pro Arg Phe
    290                 295                 300

Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser
            340                 345                 350

Leu Pro Ser Gly Asp Thr Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

Gln Lys Val Glu Glu Met Val Gln Asn His Met Asn Tyr Ser Leu Gln
    370                 375                 380
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```
Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
385                 390                 395                 400

Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
                405                 410                 415

Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
            420                 425                 430

Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
        435                 440                 445

Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
    450                 455                 460

Val Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
465                 470                 475                 480

Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp
                485                 490                 495

Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala
            500                 505                 510

Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Ile Ala Met Ile
        515                 520                 525

Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asp Gln Glu Ile Ser Arg
    530                 535                 540

Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
545                 550                 555                 560

Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
                565                 570                 575

Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala
            580                 585                 590

Gly Lys Pro Ile Leu Phe
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<400> 11
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ttccctcttc ccgtctttct cctcttttcc tctcccccga ggttggcatc gaggggggca 120

aattcgggcg gcggcgccgg gcgcagcgca ggggtcacaa cgacggcgac ggctgacggt 180

tggaagggca ggcttccttc gcccctcgac ctccttcccc ggtccgcttg gtgtcaggcg 240

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cggcggcggc ggcggcggcg gcgcggcggg cggactccat ccctcctccc gctccctcct 300

gcaccggagc gggcactcct tccttcgcca tccccccgacc cttcaccccg gggactgggc 360

gcctccaccg gcgcagctca gggagcgggg gccggtctcc tgctcggctg tcgcgcctcc 420

atg tcg gat aac cag agc tgg aac tcg tcg ggc tcg gag gag gat ccg   468
Met Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro
  1               5                  10                  15

gag acg gag tcc ggg ccg ccg gtg gag cgc tgc gga gtc ctc aac aag   516
Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Asn Lys
             20                  25                  30

tgg aca aac tat att cat ggg tgg cag gat cgc tgg gta gtt ttg aaa   564
Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
         35                  40                  45

aat aac act ctg agt tac tac aaa tct gaa gat gag aca gag tat ggc   612
Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
     50                  55                  60

tgc aga gga tcc atc tgt ctt agc aag gct gtc atc acg cct cat gat   660
Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80

ttt gat gaa tgc cga ttt gat att agt gta aat gat agt gtt tgg tat   708
Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
                 85                  90                  95

ctt cgt gct caa gat cca gat cac aga cag cag tgg ata gat gcc att   756
Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
            100                 105                 110

gaa cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt   804
Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

cga cat ggc tcc atg gta tca ttg gta tcc gga gca agt ggc tat tct   852
Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

gca aca tcc acc tcc tca ttc aag aag ggc cac agt tta cgt gag aaa   900
Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

ctg gct gaa atg gaa acc ttt aga gat ata ctg tgt aga caa gtt gat   948
Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

acc cta cag aag ttc ttt gat gcc tgt gct gat gct gtc tcc aag gat   996
Thr Leu Gln Lys Phe Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190

gaa ttt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct   1044
Glu Phe Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

acg aca cgt tct gat gga gac ttc ttg cat aat acc aat ggc aat aag   1092
Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
    210                 215                 220
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gaa aag gta ttt cca cat gta aca cca aaa gga att aat ggt ata gac   1140
Glu Lys Val Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240

ttt aaa ggt gag gcg ata act ttt aaa gca act act gcc gga atc ctt   1188
Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

gct aca ctt tct cat tgt att gag ctg atg gta aaa cgt gag gac agc   1236
Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser
            260                 265                 270

tgg caa aag aga atg gac aag gaa act gag aag aga aga aga gtg gag   1284
Trp Gln Lys Arg Met Asp Lys Glu Thr Glu Lys Arg Arg Arg Val Glu
        275                 280                 285

gaa gca tac aaa aat gcc atg aca gaa ctt aag aaa aaa tcc cac ttt   1332
Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe
    290                 295                 300

gga gga cca gat tat gag gaa ggc cca aac agt ttg att aat gaa gag   1380
Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

gag ttc ttt gat gct gtt gaa gct gct ctt gac aga caa gat aaa ata   1428
Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

gaa gaa cag tcg cag agt gaa aag gtc agg tta cat tgg tct act tca   1476
Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Ser Thr Ser
            340                 345                 350

atg cca tct gga gat gcc ttt tct tct gtg ggg act cat aga ttt gtc   1524
Met Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

caa aag gtt gaa gag atg gtg cag aac cac atg acc tat tca ttg cag   1572
Gln Lys Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln
    370                 375                 380

gat gta ggt ggg gac gcc aac tgg cag ttg gtt gta gaa gaa ggg gag   1620
Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
385                 390                 395                 400

atg aag gta tat aga aga gaa gta gaa gaa aat ggg att gtt ctg gat   1668
Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
                405                 410                 415

cct ttg aaa gct acc cat gca gtt aaa ggc gtt aca gga cac gag gtc   1716
Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
            420                 425                 430

tgc aat tac ttc tgg aat gtt gat gtt cgc aat gat tgg gaa aca act   1764
Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
        435                 440                 445

ata gaa aac ttt cat gtg gtg gaa aca tta gct gat aat gca atc atc   1812
Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
    450                 455                 460
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att tat caa acg cac aag aga gtg tgg cca gcc tct cag cgg gat gtc   1860
Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
465                 470                 475                 480

tta tat ctg tct gcc att cga aag ata cca gct ttg aat gaa aat gac   1908
Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp
                485                 490                 495

ccg gag act tgg ata gtt tgt aat ttt tct gta gat cac agc agt gct   1956
Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Ser Ser Ala
            500                 505                 510

cct cta aac aat cga tgt gtc cgt gcc aaa ata aac gtt gct atg att   2004
Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile
        515                 520                 525

tgt cag acc ttg gtg agc ccc cca gag gga aac cag gag att agc agg   2052
Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg
    530                 535                 540

gac aac att cta tgc aag att aca tac gtg gcc aat gta aac cct gga   2100
Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
545                 550                 555                 560

gga tgg gcc cca gcc tca gtg tta cgg gca gtg gca aag cga gaa tat   2148
Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
                565                 570                 575

cca aag ttt cta aag cgt ttt act tct tac gta caa gaa aaa act gca   2196
Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala
            580                 585                 590

gga aaa cct att ttg ttc tagtattaac agtgactgaa gcaaggctgt          2244
Gly Lys Pro Ile Leu Phe
        595

gtgacattcc atgttggagg aaaaaaaaaa aaaaaaaaa                        2283


<210> 12
<211> 598
<212> PRT
<213> Bos taurus

<400> 12
Met Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro
  1               5                  10                  15

Glu Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Asn Lys
             20                  25                  30

Trp Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys
         35                  40                  45

Asn Asn Thr Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly
     50                  55                  60

Cys Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp
 65                  70                  75                  80

Phe Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr
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                85                  90                  95

Leu Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile
            100                 105                 110

Glu Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg
        115                 120                 125

Arg His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser
    130                 135                 140

Ala Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys
145                 150                 155                 160

Leu Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp
                165                 170                 175

Thr Leu Gln Lys Phe Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp
            180                 185                 190

Glu Phe Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro
        195                 200                 205

Thr Thr Arg Ser Asp Gly Asp Phe Leu His Asn Thr Asn Gly Asn Lys
    210                 215                 220

Glu Lys Val Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp
225                 230                 235                 240

Phe Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu
                245                 250                 255

Ala Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser
            260                 265                 270

Trp Gln Lys Arg Met Asp Lys Glu Thr Glu Lys Arg Arg Arg Val Glu
        275                 280                 285

Glu Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe
    290                 295                 300

Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu
305                 310                 315                 320

Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile
                325                 330                 335

Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Ser Thr Ser
            340                 345                 350

Met Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val
        355                 360                 365

Gln Lys Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln
    370                 375                 380

Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
385                 390                 395                 400

Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
                405                 410                 415
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```
Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
            420                 425                 430

Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
        435                 440                 445

Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
    450                 455                 460

Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
465                 470                 475                 480

Leu Tyr Leu Ser Ala Ile Arg Lys Ile Pro Ala Leu Asn Glu Asn Asp
                485                 490                 495

Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Ser Ser Ala
            500                 505                 510

Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile
        515                 520                 525

Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg
    530                 535                 540

Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
545                 550                 555                 560

Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
                565                 570                 575

Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala
            580                 585                 590

Gly Lys Pro Ile Leu Phe
        595
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<210> 13
<211> 78
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(78)

<400> 13

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ccc tat agt cgc tct tcc tcc atg tct tcc att gat cta gtc agt gcc   48
Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala
  1               5                  10                  15

tct gat gat gtt cac aga ttc agc tcc cag                           78
Ser Asp Asp Val His Arg Phe Ser Ser Gln
             20                  25
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<210> 14
<211> 26
<212> PRT
<213> Homo sapiens

<400> 14
Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala
1 5 10 15

Ser Asp Asp Val His Arg Phe Ser Ser Gln
20 25

<210> 15
<211> 2034
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPBPR3

<220>
<221> CDS
<222> (10)..(990)

<400> 15
gaattcacc atg gcc cca cta gcc gac tac aag gac gac gat gac aag atg 51
Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met
1 5 10

tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag 99
Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
15 20 25 30

acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg 147
Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
35 40 45

aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat 195
Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
50 55 60

aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc 243
Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
65 70 75

aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt 291
Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe
80 85 90

gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt 339
Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu
95 100 105 110

cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa 387
Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu
115 120 125

cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga 435
Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg
130 135 140

cat ggc tca atg gtg tcc ctg gtg tct gga gca agt ggc tac tct gca 483
His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala
145 150 155

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aca tcc acc tct tca ttc aag aaa ggc cac agt tta cgt gag aag ttg   531
Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu
    160                 165                 170

gct gaa atg gaa aca ttt aga gac atc tta tgt aga caa gtt gac acg   579
Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr
175                 180                 185                 190

cta cag aag tac ttt gat gcc tgt gct gat gct gtc tct aag gat gaa   627
Leu Gln Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu
                195                 200                 205

ctt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct aca   675
Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr
            210                 215                 220

acg cgt tct gat ggt gac ttc ttg cat agt acc aac ggc aat aaa gaa   723
Thr Arg Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu
        225                 230                 235

aag tta ttt cca cat gtg aca cca aaa gga att aat ggt ata gac ttt   771
Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe
    240                 245                 250

aaa ggg gaa gcg ata act ttt aaa gca act act gct gga atc ctt gca   819
Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala
255                 260                 265                 270

aca ctt tct cat tgt att gaa cta atg gtt aaa cgt gag gac agc tgg   867
Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp
                275                 280                 285

cag aag aga ctg gat aag gaa act gag aag aaa aga aga aca gag gaa   915
Gln Lys Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu
            290                 295                 300

gca tat aaa aat gca atg aca gaa cga aaa aat ccc act ttg gag gac   963
Ala Tyr Lys Asn Ala Met Thr Glu Arg Lys Asn Pro Thr Leu Glu Asp
        305                 310                 315

cag att atg aag aag gcc cta aca gtc tgattaatga agaagagttc        1010
Gln Ile Met Lys Lys Ala Leu Thr Val
    320                 325

tttgatgctg ttgaagctgc tcttgacaga caagataaaa tagaagaaca gtcacagagt 1070

gaaaaggtga gattacattg gcctacatcc ttgccctctg gagatgcctt ttcttctgtg 1130

gggacacata gatttgtcca aaagccctat agtcgctctt cctccatgtc ttccattgat 1190

ctagtcagtg cctctgatga tgttcacaga ttcagctccc aggttgaaga gatggtgcag 1250

aaccacatga cttactcatt acaggatgta ggcggagatg ccaattggca gttggttgta 1310

gaagaaggag aaatgaaggt atacagaaga gaagtagaag aaaatgggat tgttctggat 1370

cctttaaaag ctacccatgc agttaaaggc gtcacaggac atgaagtctg caattatttc 1430

tggaatgttg acgttcgcaa tgactgggaa acaactatag aaaactttca tgtggtggaa 1490
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acattagctg ataatgcaat catcatttat caaacacaca agagggtgtg gcctgcttct 1550

cagcgagacg tattatatct ttctgtcatt cgaaagatac cagccttgac tgaaaatgac 1610

cctgaaactt ggatagtttg taatttttct gtggatcatg acagtgctcc tctaaacaac 1670

cgatgtgtcc gtgccaaaat aaatgttgct atgatttgtc aaaccttggt aagcccacca 1730

gagggaaacc aggaaattag cagggacaac attctatgca agattacata tgtagctaat 1790

gtgaaccctg gaggatgggc accagcctca gtgttaaggg cagtggcaaa gcgagagtat 1850

cctaaatttc taaaacgttt tacttcttac gtccaagaaa aaactgcagg aaagcctatt 1910

ttgttctagt attaacaggt actagaagat atgttttatc ttttttaac tttatttgac 1970

taatatgact gtcaatacta aaatttagtt gttgaaagta tttactatgt tttttccgga 2030

attc 2034

<210> 16
<211> 327
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPBPR3

<400> 16
Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
1 5 10 15

Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
20 25 30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
35 40 45

Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
50 55 60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
65 70 75 80

Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
85 90 95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
100 105 110

Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
115 120 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
130 135 140

Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser
145 150 155 160

Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu

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                165                 170                 175

Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln
            180                 185                 190

Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln
        195                 200                 205

Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg
    210                 215                 220

Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu
225                 230                 235                 240

Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly
                245                 250                 255

Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu
            260                 265                 270

Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys
        275                 280                 285

Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr
    290                 295                 300

Lys Asn Ala Met Thr Glu Arg Lys Asn Pro Thr Leu Glu Asp Gln Ile
305                 310                 315                 320

Met Lys Lys Ala Leu Thr Val
                325
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<210> 17
<211> 1978
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: FLAG-GPBPDNLS

<220>
<221> CDS
<222> (10)..(1860)

<400> 17

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gaattcacc atg gcc cca cta gcc gac tac aag gac gac gat gac aag atg 51
          Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met
            1               5                  10

tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag   99
Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
 15                  20                  25                  30

acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg   147
Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
                 35                  40                  45

aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat   195
Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
            50                  55                  60
```

```
aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc   243
Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
         65                  70                  75

aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt   291
Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe
     80                  85                  90

gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt   339
Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu
 95                 100                 105                 110

cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa   387
Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu
                115                 120                 125

cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga   435
Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg
            130                 135                 140

cat ggc tca atg gtg tcc ctg gtg tct gga gca agt ggc tac tct gca   483
His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala
        145                 150                 155

aca tcc acc tct tca ttc aag aaa ggc cac agt tta cgt gag aag ttg   531
Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu
    160                 165                 170

gct gaa atg gaa aca ttt aga gac atc tta tgt aga caa gtt gac acg   579
Ala Glu Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr
175                 180                 185                 190

cta cag aag tac ttt gat gcc tgt gct gat gct gtc tct aag gat gaa   627
Leu Gln Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu
                195                 200                 205

ctt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct aca   675
Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr
            210                 215                 220

acg cgt tct gat ggt gac ttc ttg cat agt acc aac ggc aat aaa gaa   723
Thr Arg Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu
        225                 230                 235

aag tta ttt cca cat gtg aca cca aaa gga att aat ggt ata gac ttt   771
Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe
    240                 245                 250

aaa ggg gaa gcg ata act ttt aaa gca act act gct gga atc ctt gca   819
Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala
255                 260                 265                 270

aca ctt tct cat tgt att gaa cta atg gtt aaa cgt gag gac agc tgg   867
Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp
                275                 280                 285

cag aag aga ctg gat aag gaa act gag cac ttt gga gga cca gat tat   915
Gln Lys Arg Leu Asp Lys Glu Thr Glu His Phe Gly Gly Pro Asp Tyr
            290                 295                 300
```

```
gaa gaa ggc cct aac agt ctg att aat gaa gaa gag ttc ttt gat gct   963
Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala
        305                 310                 315

gtt gaa gct gct ctt gac aga caa gat aaa ata gaa gaa cag tca cag   1011
Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln
    320                 325                 330

agt gaa aag gtg aga tta cat tgg cct aca tcc ttg ccc tct gga gat   1059
Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp
335                 340                 345                 350

gcc ttt tct tct gtg ggg aca cat aga ttt gtc caa aag ccc tat agt   1107
Ala Phe Ser Ser Val Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser
                355                 360                 365

cgc tct tcc tcc atg tct tcc att gat cta gtc agt gcc tct gat gat   1155
Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp
            370                 375                 380

gtt cac aga ttc agc tcc cag gtt gaa gag atg gtg cag aac cac atg   1203
Val His Arg Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn His Met
        385                 390                 395

act tac tca tta cag gat gta ggc gga gat gcc aat tgg cag ttg gtt   1251
Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val
    400                 405                 410

gta gaa gaa gga gaa atg aag gta tac aga aga gaa gta gaa gaa aat   1299
Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn
415                 420                 425                 430

ggg att gtt ctg gat cct tta aaa gct acc cat gca gtt aaa ggc gtc   1347
Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly Val
                435                 440                 445

aca gga cat gaa gtc tgc aat tat ttc tgg aat gtt gac gtt cgc aat   1395
Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn
            450                 455                 460

gac tgg gaa aca act ata gaa aac ttt cat gtg gtg gaa aca tta gct   1443
Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu Ala
        465                 470                 475

gat aat gca atc atc att tat caa aca cac aag agg gtg tgg cct gct   1491
Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala
    480                 485                 490

tct cag cga gac gta tta tat ctt tct gtc att cga aag ata cca gcc   1539
Ser Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala
495                 500                 505                 510

ttg act gaa aat gac cct gaa act tgg ata gtt tgt aat ttt tct gtg   1587
Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val
                515                 520                 525

gat cat gac agt gct cct cta aac aac cga tgt gtc cgt gcc aaa ata   1635
Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile
            530                 535                 540

aat gtt gct atg att tgt caa acc ttg gta agc cca cca gag gga aac   1683
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Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn
        545                 550                 555

cag gaa att agc agg gac aac att cta tgc aag att aca tat gta gct   1731
Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala
    560                 565                 570

aat gtg aac cct gga gga tgg gca cca gcc tca gtg tta agg gca gtg   1779
Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val
575                 580                 585                 590

gca aag cga gag tat cct aaa ttt cta aaa cgt ttt act tct tac gtc   1827
Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val
                595                 600                 605

caa gaa aaa act gca gga aag cct att ttg ttc tagtattaac aggtactaga 1880
Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
            610                 615

agatatgttt tatctttttt taactttatt tgactaatat gactgtcaat actaaaattt 1940

agttgttgaa agtatttact atgttttttc cggaattc                         1978


<210> 18
<211> 617
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: FLAG-GPBPDNLS

<400> 18
Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
  1               5                  10                  15

Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
             20                  25                  30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
         35                  40                  45

Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
     50                  55                  60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
 65                  70                  75                  80

Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
                 85                  90                  95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
            100                 105                 110

Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
        115                 120                 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
    130                 135                 140

Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser
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145 150 155 160

Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu
165 170 175

Met Glu Thr Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln
180 185 190

Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln
195 200 205

Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg
210 215 220

Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu
225 230 235 240

Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly
245 250 255

Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu
260 265 270

Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys
275 280 285

Arg Leu Asp Lys Glu Thr Glu His Phe Gly Gly Pro Asp Tyr Glu Glu
290 295 300

Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu
305 310 315 320

Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu
325 330 335

Lys Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala Phe
340 345 350

Ser Ser Val Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser
355 360 365

Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val His
370 375 380

Arg Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn His Met Thr Tyr
385 390 395 400

Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu
405 410 415

Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile
420 425 430

Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly
435 440 445

His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp
450 455 460

Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn
465 470 475 480

```
Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln
                485                 490                 495

Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr
            500                 505                 510

Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His
        515                 520                 525

Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val
    530                 535                 540

Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu
545                 550                 555                 560

Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val
                565                 570                 575

Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys
            580                 585                 590

Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu
        595                 600                 605

Lys Thr Ala Gly Lys Pro Ile Leu Phe
    610                 615
```

<210> 19
<211> 1975
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: FLAG-GPBPDSXY

<220>
<221> CDS
<222> (10)..(1857)

<400> 19

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gaattcacc atg gcc cca cta gcc gac tac aag gac gac gat gac aag atg 51
          Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met
            1               5                  10

tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag   99
Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
 15                  20                  25                  30

acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg   147
Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
                 35                  40                  45

aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat   195
Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
             50                  55                  60

aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc   243
Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
         65                  70                  75
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aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt   291
Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe
     80                  85                  90

gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt   339
Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu
 95                 100                 105                 110

cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa   387
Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu
                115                 120                 125

cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga   435
Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg
            130                 135                 140

cat ggc aaa ggc cac agt tta cgt gag aag ttg gct gaa atg gaa aca   483
His Gly Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr
        145                 150                 155

ttt aga gac atc tta tgt aga caa gtt gac acg cta cag aag tac ttt   531
Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe
    160                 165                 170

gat gcc tgt gct gat gct gtc tct aag gat gaa ctt caa agg gat aaa   579
Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys
175                 180                 185                 190

gtg gta gaa gat gat gaa gat gac ttt cct aca acg cgt tct gat ggt   627
Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly
                195                 200                 205

gac ttc ttg cat agt acc aac ggc aat aaa gaa aag tta ttt cca cat   675
Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His
            210                 215                 220

gtg aca cca aaa gga att aat ggt ata gac ttt aaa ggg gaa gcg ata   723
Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile
        225                 230                 235

act ttt aaa gca act act gct gga atc ctt gca aca ctt tct cat tgt   771
Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys
    240                 245                 250

att gaa cta atg gtt aaa cgt gag gac agc tgg cag aag aga ctg gat   819
Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp
255                 260                 265                 270

aag gaa act gag aag aaa aga aga aca gag gaa gca tat aaa aat gca   867
Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr Lys Asn Ala
                275                 280                 285

atg aca gaa ctt aag aaa aaa tcc cac ttt gga gga cca gat tat gaa   915
Met Thr Glu Leu Lys Lys Lys Ser His Phe Gly Gly Pro Asp Tyr Glu
            290                 295                 300

gaa ggc cct aac agt ctg att aat gaa gaa gag ttc ttt gat gct gtt   963
Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val
        305                 310                 315
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gaa gct gct ctt gac aga caa gat aaa ata gaa gaa cag tca cag agt   1011
Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser
    320                 325                 330

gaa aag gtg aga tta cat tgg cct aca tcc ttg ccc tct gga gat gcc   1059
Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala
335                 340                 345                 350

ttt tct tct gtg ggg aca cat aga ttt gtc caa aag ccc tat agt cgc   1107
Phe Ser Ser Val Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg
                355                 360                 365

tct tcc tcc atg tct tcc att gat cta gtc agt gcc tct gat gat gtt   1155
Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val
            370                 375                 380

cac aga ttc agc tcc cag gtt gaa gag atg gtg cag aac cac atg act   1203
His Arg Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn His Met Thr
        385                 390                 395

tac tca tta cag gat gta ggc gga gat gcc aat tgg cag ttg gtt gta   1251
Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val
    400                 405                 410

gaa gaa gga gaa atg aag gta tac aga aga gaa gta gaa gaa aat ggg   1299
Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly
415                 420                 425                 430

att gtt ctg gat cct tta aaa gct acc cat gca gtt aaa ggc gtc aca   1347
Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr
                435                 440                 445

gga cat gaa gtc tgc aat tat ttc tgg aat gtt gac gtt cgc aat gac   1395
Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp
            450                 455                 460

tgg gaa aca act ata gaa aac ttt cat gtg gtg gaa aca tta gct gat   1443
Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp
        465                 470                 475

aat gca atc atc att tat caa aca cac aag agg gtg tgg cct gct tct   1491
Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser
    480                 485                 490

cag cga gac gta tta tat ctt tct gtc att cga aag ata cca gcc ttg   1539
Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu
495                 500                 505                 510

act gaa aat gac cct gaa act tgg ata gtt tgt aat ttt tct gtg gat   1587
Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp
                515                 520                 525

cat gac agt gct cct cta aac aac cga tgt gtc cgt gcc aaa ata aat   1635
His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn
            530                 535                 540

gtt gct atg att tgt caa acc ttg gta agc cca cca gag gga aac cag   1683
Val Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln
        545                 550                 555

gaa att agc agg gac aac att cta tgc aag att aca tat gta gct aat   1731
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Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn
    560                 565                 570

gtg aac cct gga gga tgg gca cca gcc tca gtg tta agg gca gtg gca   1779
Val Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala
575                 580                 585                 590

aag cga gag tat cct aaa ttt cta aaa cgt ttt act tct tac gtc caa   1827
Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln
                595                 600                 605

gaa aaa act gca gga aag cct att ttg ttc tagtattaac aggtactaga     1877
Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
            610                 615

agatatgttt tatcttttt taactttatt tgactaatat gactgtcaat actaaaattt 1937

agttgttgaa agtatttact atgtttttc cggaattc                          1975


<210> 20
<211> 616
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: FLAG-GPBPDSXY

<400> 20
Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
  1               5                  10                  15

Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
             20                  25                  30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
         35                  40                  45

Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
     50                  55                  60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
 65                  70                  75                  80

Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
                 85                  90                  95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
            100                 105                 110

Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
        115                 120                 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
    130                 135                 140

Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg
145                 150                 155                 160

Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Ala
                165                 170                 175
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Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val
            180                 185                 190

Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe
        195                 200                 205

Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr
    210                 215                 220

Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe
225                 230                 235                 240

Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu
                245                 250                 255

Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp Lys Glu
            260                 265                 270

Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr Lys Asn Ala Met Thr
        275                 280                 285

Glu Leu Lys Lys Lys Ser His Phe Gly Gly Pro Asp Tyr Glu Glu Gly
    290                 295                 300

Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala
305                 310                 315                 320

Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys
                325                 330                 335

Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala Phe Ser
            340                 345                 350

Ser Val Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser Ser
        355                 360                 365

Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val His Arg
    370                 375                 380

Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser
385                 390                 395                 400

Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu
                405                 410                 415

Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val
            420                 425                 430

Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His
        435                 440                 445

Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu
    450                 455                 460

Thr Thr Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala
465                 470                 475                 480

Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg
                485                 490                 495
```

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Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu
            500                 505                 510

Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp
        515                 520                 525

Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala
    530                 535                 540

Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile
545                 550                 555                 560

Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn
                565                 570                 575

Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg
            580                 585                 590

Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys
        595                 600                 605

Thr Ala Gly Lys Pro Ile Leu Phe
    610                 615


<210> 21
<211> 1915
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      FLAG-GPBPDSXY/NLS

<220>
<221> CDS
<222> (10)..(1797)

<400> 21
gaattcacc atg gcc cca cta gcc gac tac aag gac gac gat gac aag atg 51
          Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met
            1               5                  10

tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag   99
Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
 15                  20                  25                  30

acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg   147
Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
                 35                  40                  45

aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat   195
Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
            50                  55                  60

aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc   243
Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
        65                  70                  75

aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt   291
Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe
```

```
     80                  85                  90

gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt   339
Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu
 95                  100                 105                 110

cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa   387
Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu
                115                 120                 125

cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga   435
Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg
            130                 135                 140

cat ggc aaa ggc cac agt tta cgt gag aag ttg gct gaa atg gaa aca   483
His Gly Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr
        145                 150                 155

ttt aga gac atc tta tgt aga caa gtt gac acg cta cag aag tac ttt   531
Phe Arg Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe
    160                 165                 170

gat gcc tgt gct gat gct gtc tct aag gat gaa ctt caa agg gat aaa   579
Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys
175                 180                 185                 190

gtg gta gaa gat gat gaa gat gac ttt cct aca acg cgt tct gat ggt   627
Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly
                195                 200                 205

gac ttc ttg cat agt acc aac ggc aat aaa gaa aag tta ttt cca cat   675
Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His
            210                 215                 220

gtg aca cca aaa gga att aat ggt ata gac ttt aaa ggg gaa gcg ata   723
Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile
        225                 230                 235

act ttt aaa gca act act gct gga atc ctt gca aca ctt tct cat tgt   771
Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys
    240                 245                 250

att gaa cta atg gtt aaa cgt gag gac agc tgg cag aag aga ctg gat   819
Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp
255                 260                 265                 270

aag gaa act gag cac ttt gga gga cca gat tat gaa gaa ggc cct aac   867
Lys Glu Thr Glu His Phe Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn
                275                 280                 285

agt ctg att aat gaa gaa gag ttc ttt gat gct gtt gaa gct gct ctt   915
Ser Leu Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala Ala Leu
            290                 295                 300

gac aga caa gat aaa ata gaa gaa cag tca cag agt gaa aag gtg aga   963
Asp Arg Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys Val Arg
        305                 310                 315

tta cat tgg cct aca tcc ttg ccc tct gga gat gcc ttt tct tct gtg   1011
Leu His Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala Phe Ser Ser Val
    320                 325                 330
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ggg aca cat aga ttt gtc caa aag ccc tat agt cgc tct tcc tcc atg   1059
Gly Thr His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met
335                 340                 345                 350

tct tcc att gat cta gtc agt gcc tct gat gat gtt cac aga ttc agc   1107
Ser Ser Ile Asp Leu Val Ser Ala Ser Asp Asp Val His Arg Phe Ser
                355                 360                 365

tcc cag gtt gaa gag atg gtg cag aac cac atg act tac tca tta cag   1155
Ser Gln Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln
            370                 375                 380

gat gta ggc gga gat gcc aat tgg cag ttg gtt gta gaa gaa gga gaa   1203
Asp Val Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu
        385                 390                 395

atg aag gta tac aga aga gaa gta gaa gaa aat ggg att gtt ctg gat   1251
Met Lys Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp
    400                 405                 410

cct tta aaa gct acc cat gca gtt aaa ggc gtc aca gga cat gaa gtc   1299
Pro Leu Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val
415                 420                 425                 430

tgc aat tat ttc tgg aat gtt gac gtt cgc aat gac tgg gaa aca act   1347
Cys Asn Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr
                435                 440                 445

ata gaa aac ttt cat gtg gtg gaa aca tta gct gat aat gca atc atc   1395
Ile Glu Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile
            450                 455                 460

att tat caa aca cac aag agg gtg tgg cct gct tct cag cga gac gta   1443
Ile Tyr Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val
        465                 470                 475

tta tat ctt tct gtc att cga aag ata cca gcc ttg act gaa aat gac   1491
Leu Tyr Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp
    480                 485                 490

cct gaa act tgg ata gtt tgt aat ttt tct gtg gat cat gac agt gct   1539
Pro Glu Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala
495                 500                 505                 510

cct cta aac aac cga tgt gtc cgt gcc aaa ata aat gtt gct atg att   1587
Pro Leu Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile
                515                 520                 525

tgt caa acc ttg gta agc cca cca gag gga aac cag gaa att agc agg   1635
Cys Gln Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg
            530                 535                 540

gac aac att cta tgc aag att aca tat gta gct aat gtg aac cct gga   1683
Asp Asn Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly
        545                 550                 555

gga tgg gca cca gcc tca gtg tta agg gca gtg gca aag cga gag tat   1731
Gly Trp Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr
    560                 565                 570
```

```
cct aaa ttt cta aaa cgt ttt act tct tac gtc caa gaa aaa act gca   1779
Pro Lys Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala
575                 580                 585                 590

gga aag cct att ttg ttc tagtattaac aggtactaga agatatgttt          1827
Gly Lys Pro Ile Leu Phe
                595

tatctttttt taactttatt tgactaatat gactgtcaat actaaaattt agttgttgaa 1887

agtatttact atgttttttc cggaattc                                    1915
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<210> 22
<211> 596
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
FLAG-GPBPDSXY/NLS

<400> 22

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Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
  1               5                  10                  15

Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
             20                  25                  30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
         35                  40                  45

Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
     50                  55                  60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
 65                  70                  75                  80

Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
                 85                  90                  95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
            100                 105                 110

Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
        115                 120                 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
    130                 135                 140

Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu Met Glu Thr Phe Arg
145                 150                 155                 160

Asp Ile Leu Cys Arg Gln Val Asp Thr Leu Gln Lys Tyr Phe Asp Ala
                165                 170                 175

Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln Arg Asp Lys Val Val
            180                 185                 190

Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg Ser Asp Gly Asp Phe
        195                 200                 205
```

```
Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu Phe Pro His Val Thr
    210                 215                 220

Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly Glu Ala Ile Thr Phe
225                 230                 235                 240

Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu Ser His Cys Ile Glu
                245                 250                 255

Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys Arg Leu Asp Lys Glu
            260                 265                 270

Thr Glu His Phe Gly Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu
        275                 280                 285

Ile Asn Glu Glu Glu Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg
    290                 295                 300

Gln Asp Lys Ile Glu Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His
305                 310                 315                 320

Trp Pro Thr Ser Leu Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr
                325                 330                 335

His Arg Phe Val Gln Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser
            340                 345                 350

Ile Asp Leu Val Ser Ala Ser Asp Asp Val His Arg Phe Ser Ser Gln
        355                 360                 365

Val Glu Glu Met Val Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val
    370                 375                 380

Gly Gly Asp Ala Asn Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys
385                 390                 395                 400

Val Tyr Arg Arg Glu Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu
                405                 410                 415

Lys Ala Thr His Ala Val Lys Gly Val Thr Gly His Glu Val Cys Asn
            420                 425                 430

Tyr Phe Trp Asn Val Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu
        435                 440                 445

Asn Phe His Val Val Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr
    450                 455                 460

Gln Thr His Lys Arg Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr
465                 470                 475                 480

Leu Ser Val Ile Arg Lys Ile Pro Ala Leu Thr Glu Asn Asp Pro Glu
                485                 490                 495

Thr Trp Ile Val Cys Asn Phe Ser Val Asp His Asp Ser Ala Pro Leu
            500                 505                 510

Asn Asn Arg Cys Val Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln
        515                 520                 525
```

```
Thr Leu Val Ser Pro Pro Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn
    530                 535                 540

Ile Leu Cys Lys Ile Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp
545                 550                 555                 560

Ala Pro Ala Ser Val Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys
                565                 570                 575

Phe Leu Lys Arg Phe Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys
            580                 585                 590

Pro Ile Leu Phe
        595
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<210> 23
<211> 2038
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPBP-D169A

<220>
<221> CDS
<222> (10)..(1920)

<400> 23

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gaattcacc atg gcc cca cta gcc gac tac aag gac gac gat gac aag atg 51
          Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met
            1               5                  10

tcg gat aat cag agc tgg aac tcg tcg ggc tcg gag gag gat cca gag   99
Ser Asp Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu
 15                  20                  25                  30

acg gag tct ggg ccg cct gtg gag cgc tgc ggg gtc ctc agt aag tgg   147
Thr Glu Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp
                 35                  40                  45

aca aac tac att cat ggg tgg cag gat cgt tgg gta gtt ttg aaa aat   195
Thr Asn Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn
             50                  55                  60

aat gct ctg agt tac tac aaa tct gaa gat gaa aca gag tat ggc tgc   243
Asn Ala Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys
         65                  70                  75

aga gga tcc atc tgt ctt agc aag gct gtc atc aca cct cac gat ttt   291
Arg Gly Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe
     80                  85                  90

gat gaa tgt cga ttt gat att agt gta aat gat agt gtt tgg tat ctt   339
Asp Glu Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu
 95                  100                 105                 110

cgt gct cag gat cca gat cat aga cag caa tgg ata gat gcc att gaa   387
Arg Ala Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu
                115                 120                 125
```

```
cag cac aag act gaa tct gga tat gga tct gaa tcc agc ttg cgt cga   435
Gln His Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg
            130                 135                 140

cat ggc tca atg gtg tcc ctg gtg tct gga gca agt ggc tac tct gca   483
His Gly Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala
        145                 150                 155

aca tcc acc tct tca ttc aag aaa ggc cac agt tta cgt gag aag ttg   531
Thr Ser Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu
    160                 165                 170

gct gaa atg gaa aca ttt aga gcc atc tta tgt aga caa gtt gac acg   579
Ala Glu Met Glu Thr Phe Arg Ala Ile Leu Cys Arg Gln Val Asp Thr
175                 180                 185                 190

cta cag aag tac ttt gat gcc tgt gct gat gct gtc tct aag gat gaa   627
Leu Gln Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu
                195                 200                 205

ctt caa agg gat aaa gtg gta gaa gat gat gaa gat gac ttt cct aca   675
Leu Gln Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr
            210                 215                 220

acg cgt tct gat ggt gac ttc ttg cat agt acc aac ggc aat aaa gaa   723
Thr Arg Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu
        225                 230                 235

aag tta ttt cca cat gtg aca cca aaa gga att aat ggt ata gac ttt   771
Lys Leu Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe
    240                 245                 250

aaa ggg gaa gcg ata act ttt aaa gca act act gct gga atc ctt gca   819
Lys Gly Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala
255                 260                 265                 270

aca ctt tct cat tgt att gaa cta atg gtt aaa cgt gag gac agc tgg   867
Thr Leu Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp
                275                 280                 285

cag aag aga ctg gat aag gaa act gag aag aaa aga aga aca gag gaa   915
Gln Lys Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu
            290                 295                 300

gca tat aaa aat gca atg aca gaa ctt aag aaa aaa tcc cac ttt gga   963
Ala Tyr Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe Gly
        305                 310                 315

gga cca gat tat gaa gaa ggc cct aac agt ctg att aat gaa gaa gag   1011
Gly Pro Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu
    320                 325                 330

ttc ttt gat gct gtt gaa gct gct ctt gac aga caa gat aaa ata gaa   1059
Phe Phe Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu
335                 340                 345                 350

gaa cag tca cag agt gaa aag gtg aga tta cat tgg cct aca tcc ttg   1107
Glu Gln Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu
                355                 360                 365

ccc tct gga gat gcc ttt tct tct gtg ggg aca cat aga ttt gtc caa   1155
```

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Pro Ser Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val Gln
            370                 375                 380

aag ccc tat agt cgc tct tcc tcc atg tct tcc att gat cta gtc agt   1203
Lys Pro Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser
        385                 390                 395

gcc tct gat gat gtt cac aga ttc agc tcc cag gtt gaa gag atg gtg   1251
Ala Ser Asp Asp Val His Arg Phe Ser Ser Gln Val Glu Glu Met Val
    400                 405                 410

cag aac cac atg act tac tca tta cag gat gta ggc gga gat gcc aat   1299
Gln Asn His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn
415                 420                 425                 430

tgg cag ttg gtt gta gaa gaa gga gaa atg aag gta tac aga aga gaa   1347
Trp Gln Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu
                435                 440                 445

gta gaa gaa aat ggg att gtt ctg gat cct tta aaa gct acc cat gca   1395
Val Glu Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala
            450                 455                 460

gtt aaa ggc gtc aca gga cat gaa gtc tgc aat tat ttc tgg aat gtt   1443
Val Lys Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val
        465                 470                 475

gac gtt cgc aat gac tgg gaa aca act ata gaa aac ttt cat gtg gtg   1491
Asp Val Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val
    480                 485                 490

gaa aca tta gct gat aat gca atc atc att tat caa aca cac aag agg   1539
Glu Thr Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg
495                 500                 505                 510

gtg tgg cct gct tct cag cga gac gta tta tat ctt tct gtc att cga   1587
Val Trp Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg
                515                 520                 525

aag ata cca gcc ttg act gaa aat gac cct gaa act tgg ata gtt tgt   1635
Lys Ile Pro Ala Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys
            530                 535                 540

aat ttt tct gtg gat cat gac agt gct cct cta aac aac cga tgt gtc   1683
Asn Phe Ser Val Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val
        545                 550                 555

cgt gcc aaa ata aat gtt gct atg att tgt caa acc ttg gta agc cca   1731
Arg Ala Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser Pro
    560                 565                 570

cca gag gga aac cag gaa att agc agg gac aac att cta tgc aag att   1779
Pro Glu Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile
575                 580                 585                 590

aca tat gta gct aat gtg aac cct gga gga tgg gca cca gcc tca gtg   1827
Thr Tyr Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser Val
                595                 600                 605

tta agg gca gtg gca aag cga gag tat cct aaa ttt cta aaa cgt ttt   1875
Leu Arg Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe
```

610 615 620

act tct tac gtc caa gaa aaa act gca gga aag cct att ttg ttc 1920
Thr Ser Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
625 630 635

tagtattaac aggtactaga agatatgttt tatcttttt taactttatt tgactaatat 1980

gactgtcaat actaaaattt agttgttgaa agtatttact atgtttttc cggaattc 2038

<210> 24
<211> 637
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPBP-D169A

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Met Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Met Ser Asp
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Asn Gln Ser Trp Asn Ser Ser Gly Ser Glu Glu Asp Pro Glu Thr Glu
20 25 30

Ser Gly Pro Pro Val Glu Arg Cys Gly Val Leu Ser Lys Trp Thr Asn
35 40 45

Tyr Ile His Gly Trp Gln Asp Arg Trp Val Val Leu Lys Asn Asn Ala
50 55 60

Leu Ser Tyr Tyr Lys Ser Glu Asp Glu Thr Glu Tyr Gly Cys Arg Gly
65 70 75 80

Ser Ile Cys Leu Ser Lys Ala Val Ile Thr Pro His Asp Phe Asp Glu
85 90 95

Cys Arg Phe Asp Ile Ser Val Asn Asp Ser Val Trp Tyr Leu Arg Ala
100 105 110

Gln Asp Pro Asp His Arg Gln Gln Trp Ile Asp Ala Ile Glu Gln His
115 120 125

Lys Thr Glu Ser Gly Tyr Gly Ser Glu Ser Ser Leu Arg Arg His Gly
130 135 140

Ser Met Val Ser Leu Val Ser Gly Ala Ser Gly Tyr Ser Ala Thr Ser
145 150 155 160

Thr Ser Ser Phe Lys Lys Gly His Ser Leu Arg Glu Lys Leu Ala Glu
165 170 175

Met Glu Thr Phe Arg Ala Ile Leu Cys Arg Gln Val Asp Thr Leu Gln
180 185 190

Lys Tyr Phe Asp Ala Cys Ala Asp Ala Val Ser Lys Asp Glu Leu Gln
195 200 205

Arg Asp Lys Val Val Glu Asp Asp Glu Asp Asp Phe Pro Thr Thr Arg
210 215 220

Ser Asp Gly Asp Phe Leu His Ser Thr Asn Gly Asn Lys Glu Lys Leu
225 230 235 240

Phe Pro His Val Thr Pro Lys Gly Ile Asn Gly Ile Asp Phe Lys Gly
245 250 255

Glu Ala Ile Thr Phe Lys Ala Thr Thr Ala Gly Ile Leu Ala Thr Leu
260 265 270

Ser His Cys Ile Glu Leu Met Val Lys Arg Glu Asp Ser Trp Gln Lys
275 280 285

Arg Leu Asp Lys Glu Thr Glu Lys Lys Arg Arg Thr Glu Glu Ala Tyr
290 295 300

Lys Asn Ala Met Thr Glu Leu Lys Lys Lys Ser His Phe Gly Gly Pro
305 310 315 320

Asp Tyr Glu Glu Gly Pro Asn Ser Leu Ile Asn Glu Glu Glu Phe Phe
325 330 335

Asp Ala Val Glu Ala Ala Leu Asp Arg Gln Asp Lys Ile Glu Glu Gln
340 345 350

Ser Gln Ser Glu Lys Val Arg Leu His Trp Pro Thr Ser Leu Pro Ser
355 360 365

Gly Asp Ala Phe Ser Ser Val Gly Thr His Arg Phe Val Gln Lys Pro
370 375 380

Tyr Ser Arg Ser Ser Ser Met Ser Ser Ile Asp Leu Val Ser Ala Ser
385 390 395 400

Asp Asp Val His Arg Phe Ser Ser Gln Val Glu Glu Met Val Gln Asn
405 410 415

His Met Thr Tyr Ser Leu Gln Asp Val Gly Gly Asp Ala Asn Trp Gln
420 425 430

Leu Val Val Glu Glu Gly Glu Met Lys Val Tyr Arg Arg Glu Val Glu
435 440 445

Glu Asn Gly Ile Val Leu Asp Pro Leu Lys Ala Thr His Ala Val Lys
450 455 460

Gly Val Thr Gly His Glu Val Cys Asn Tyr Phe Trp Asn Val Asp Val
465 470 475 480

Arg Asn Asp Trp Glu Thr Thr Ile Glu Asn Phe His Val Val Glu Thr
485 490 495

Leu Ala Asp Asn Ala Ile Ile Ile Tyr Gln Thr His Lys Arg Val Trp
500 505 510

Pro Ala Ser Gln Arg Asp Val Leu Tyr Leu Ser Val Ile Arg Lys Ile
515 520 525

Pro Ala Leu Thr Glu Asn Asp Pro Glu Thr Trp Ile Val Cys Asn Phe
530 535 540

Ser Val Asp His Asp Ser Ala Pro Leu Asn Asn Arg Cys Val Arg Ala
545 550 555 560

Lys Ile Asn Val Ala Met Ile Cys Gln Thr Leu Val Ser Pro Pro Glu
565 570 575

Gly Asn Gln Glu Ile Ser Arg Asp Asn Ile Leu Cys Lys Ile Thr Tyr
580 585 590

Val Ala Asn Val Asn Pro Gly Gly Trp Ala Pro Ala Ser Val Leu Arg
595 600 605

Ala Val Ala Lys Arg Glu Tyr Pro Lys Phe Leu Lys Arg Phe Thr Ser
610 615 620

Tyr Val Gln Glu Lys Thr Ala Gly Lys Pro Ile Leu Phe
625 630 635

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<211> 12482
<212> DNA
<213> Homo sapiens

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aaacataggg aaagaaagat acatgggata aactggtgca tgagaaatga gatcttagca 180

gttggttgaa ataaatgaga acaactgagg caaactaaag aggaagaagg gcaagtggca 240

gcttaacagg agtaagatga tgagatgaag ggcagaatac cttcatggag aggaggcaaa 300

gagatataca tgatatgttc ttaggaacat aactgaagca aacaatgata ttatttctaa 360

ttatatataa acctgtgagt cagccttcca ggggcggcct gctaaggtag aatcattgga 420

atgatttggc cagggtttgg ataggagaga attggcagca gcgttaagat tgacccatga 480

taaataatgc tatgcaggta gcagggagtc tgactaggag caaaatcaac gaacttatcc 540

cttgcctaac atagtatctg tggagtcaga aagaagaggt taaattggga tatctgaggc 600

aagtatcagg atttgccatg tctgcggagt agtttcataa ttctaatggt tataagcact 660

aaggcgttca ctaagtgaat gttggtagtt ccaggttata ttatccattc ttgagttaca 720

aaatacactt taaaaccttc ccatcttaat attatatgtt tttttagtca cagagtgaaa 780

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cacatagatt tgtccaaaag gtaagctaat gtcagagttt actaaaagta caccttgtat 900

tgttcttcat tgttggtgga aatatctttt atttgagacg gagtctcact ctgtcaccag 960

agtggagtgc agtggcgcga tctcggctca ctacagtctc cacctcccgg gttcaagaga 1020

ttctcgtgcc tcagcctccc tggtagctgg gattacaggc atgtaccacc acacccagct 1080

aatttttgta tttttaatgg agacagtttc accatggcca ggatggtctt gatctcctga 1140

ccttgtgatc cacccacctc agcctcccag agtgctggga ttacaggcgt gagccaccat 1200

gcccagccgg aaatatcttg tagtatataa gttttctccc cttttcatta atttaagtaa 1260

tgagactgtt tttggtttta tatattgtat tccatataca tcctccaaaa cagttagaaa 1320

ttttgttctg aaaataaagt tctttcattt ttatttaagg ggaaagttgg gggtgggcaa 1380

ataaggagtg gctagtccaa aatagttaac cagaagtata tccagttata ctaaatctct 1440

ctcttctttg gggttaaatg gtattacttt gtattattgg aagcactaca ttctttttg 1500

gaatgatttt ggaacataat acataatagg tgcatgaagt cagcagttgc tgctgtgctt 1560

gtttcatata gtgctttgtt ttctcttccc tttatcttgt gtttggaagt tggtactgaa 1620

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atttcagcct gctttcccag ttcctagaaa tcttttggtt aggccactga ttttagtttt 1860

gaattttaaa tagtaacatt aagcattaaa aaggtcttcc ttgtctacta aatagttcct 1920

ctgtcaggtt tgcatgtgtc ctttactatt cacagcttgg aattttgtca tataggaggt 1980

actccagaaa gattttcaaa ctgaattgaa acaaatagaa gatactgggt tttgtatatc 2040

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atttctgttc atatgctaaa tctatggaaa tttgttttaa tttttagcat tggtaagggt 2340

ttaggaattt aagacaggaa gctggatgct tgcggtctct aaagtctgta ccctcaaaat 2400

aaaatcagat taccattgga agaagttttt tttagtgtca gcgttagttc ttttttttaat 2460

tttcttaatc ttcacatctt tgccattcaa ctttttatct ttctggtgat tgcattttat 2520

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ctcagtttaa accctggtta ggtgtatgat cccagtaagt tttctaactt ttttgtgctt 2640

catttttatg atttagctag aacctgacac ataataagtg ctcaataaat gttaccttgt 2700

attgctatta taacataatt tctttgagct aataaaagtt atctacatca ttatttttttc 2760

ctctgtgaga gtattgctat aaaagttttt aaaagtcata gtttaaagag atttctatta 2820

tttttatgtt tataaataaa gtttacatta gtttttaacc tgcaatagag aagaatatta 2880

agactttaat ttttctgact tgtacagcgt ttttctcctt gaatactctt aagaaaaaga 2940

tttagcaatt ctggatcaga aatcatccat aaccaaatat accacagtat attttacctt 3000

ttgcttgtcc atttatgcat ttttttttaa ttttacttat ttatttcga gacagggtct 3060

tgctctgttg cccaggctgg agtgcagtgg cacgatctgg gctcactgca acctccatct 3120

cccaggttca agcaattctc ctgcctcagc ctcccaagta gctgggatta caggcacgca 3180

ccactatgcc cagctaattt ttgtattctt agtaaagacg ggttttcacc atgttggcca 3240

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ataatgaatc tgtgacaaaa ctacataata ctgggagact ttggtttatt gtgctaagct 3420

ccacattgca ttaaaatcat atcacagact aatcaaaaat gcaggaatac ataggctata 3480

aatgaaagaa aatataatga cagcaaagaa agaatgtaag ccagtaataa agaatgccta 3540

agaattaggg gttcagaacc caaaccaggg ccctcactgt agtgctgtag aacagctgaa 3600

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Gly Phe Val Phe Thr
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<210> 27
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<220>
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Gly Phe Val Phe Thr
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<210> 29
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<210> 30
<211> 32
<212> DNA
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<223> Description of Artificial Sequence: ON-HNC-B-N-14m

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Ser Ser

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<212> DNA
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<400> 34
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<210> 35
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<212> DNA
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catgggtagc ttttaaag 18

<210> 37
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<223> Description of Artificial Sequence: ON-GPBP-53c

<400> 38
gaattcgaac aaaataggct ttc 23

<210> 39
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<223> Description of Artificial Sequence: ON-GPBP-56m

<400> 39
ccctatagtc gctcttc 17

<210> 40
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<400> 40
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<210> 41
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<210> 42
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Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu Phe Val Lys Val Leu
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Arg Ser Pro

<210> 44
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<212> PRT
<213> Artificial Sequence

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<210> 45
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acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct 96
Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
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tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctt 144
Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
35 40 45

ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gga act ctt 192
Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Gly Thr Leu
50 55 60

ggc agc tgc ctg cag cga ttt acc aca atg cca ttc tta ttc tgc aat 240
Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu Phe Cys Asn
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Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp
85 90 95

ctg tca aca cca gct ctg atg cca atg aac atg gct ccc att act ggc 336
Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro Ile Thr Gly

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Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys Glu Gly Pro
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gcg atc gcc ata gcc gtt cac agc caa acc act gac att cct cca tgt   432
Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile Pro Pro Cys
    130                 135                 140

cct cac ggc tgg att tct ctc tgg aaa gga ttt tca ttc atc atg aaa   480
Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe Ile Met Lys
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Ala Tyr Ser Ile Asn Cys Glu Ser Trp Gly Ile Arg Lys Asn Asn Lys
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tcg ctg tca ggt gtg cat gaa gaa aag aca ctg aag cta aaa aag aca   576
Ser Leu Ser Gly Val His Glu Glu Lys Thr Leu Lys Leu Lys Lys Thr
            180                 185                 190

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Ala Glu Leu Leu Phe Phe Ile Leu Lys Asn Lys Val Met Thr Glu His
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gct gtt att taggtatttt tctttaacca aacaatattg ctccatgatg           673
Ala Val Ile
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Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
         35                  40                  45

Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Gly Thr Leu
     50                  55                  60

Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu Phe Cys Asn
 65                  70                  75                  80

Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp
                 85                  90                  95

Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro Ile Thr Gly
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Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys Glu Gly Pro
        115                 120                 125

Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile Pro Pro Cys
    130                 135                 140

Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe Ile Met Lys
145                 150                 155                 160

Ala Tyr Ser Ile Asn Cys Glu Ser Trp Gly Ile Arg Lys Asn Asn Lys
                165                 170                 175

Ser Leu Ser Gly Val His Glu Glu Lys Thr Leu Lys Leu Lys Lys Thr
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Ala Glu Leu Leu Phe Phe Ile Leu Lys Asn Lys Val Met Thr Glu His
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<223> Description of Artificial Sequence: GPDIII

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<221> CDS
<222> (1)..(216)

<400> 47

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ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc tgg aca   48
Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
  1               5                  10                  15

acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct   96
Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
             20                  25                  30

tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctt   144
Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
         35                  40                  45

ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gat gca ctg   192
Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
     50                  55                  60

ttt gtg aag gtc ctg cga tcg cca tagccgttca cagccaaacc actgacattc  246
Phe Val Lys Val Leu Arg Ser Pro
 65                  70

ctccatgtcc tcacggctgg atttctctct ggaaaggatt ttcattcatc atgttcacaa 306

gtgcaggttc tgagggcacc gggcaagcac tggcctcccc tggctcctgc ctggaagaat 366
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tccgagccag cccatttcta gaatgtcatg gaagaggaac gtgcaactac tattcaaatt 426

cctacagttt ctggctggct tcattaaacc cagaaagaat gttcagaaag cctattccat 486

caactgtgaa agctggggaa ttagaaaaaa taataagtcg ctgtcaggtg tgcatgaaga 546

aaagacactg aagctaaaaa agacagcaga actgctattt ttcatcctaa agaacaaagt 606

aatgacagaa catgctgtta tttaggtatt tttctttaac caaacaatat tgctccatga 666

tgacttagta caaa 680

<210> 48
<211> 72
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPDIII

<400> 48
Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
1 5 10 15

Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
20 25 30

Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
35 40 45

Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
50 55 60

Phe Val Lys Val Leu Arg Ser Pro
65 70

<210> 49
<211> 392
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPDIII-IV-V

<220>
<221> CDS
<222> (1)..(207)

<400> 49
ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc tgg aca 48
Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
1 5 10 15

acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct 96
Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
20 25 30

tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctt 144
Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu

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        35                  40                  45

ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gaa agc cta   192
Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Glu Ser Leu
     50                  55                  60

ttc cat caa ctg tga aagctgggga attagaaaaa ataataagtc gctgtcaggt   247
Phe His Gln Leu
 65

gtgcatgaag aaaagacact gaagctaaaa aagacagcag aactgctatt tttcatccta 307

aagaacaaag taatgacaga acatgctgtt atttaggtat ttttctttaa ccaaacaata 367

ttgctccatg atgacttagt acaaa                                       392


<210> 50
<211> 68
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPDIII-IV-V

<400> 50
Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
  1               5                  10                  15

Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
             20                  25                  30

Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
         35                  40                  45

Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Glu Ser Leu
     50                  55                  60

Phe His Gln Leu
 65


<210> 51
<211> 507
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPDIII-V

<220>
<221> CDS
<222> (1)..(216)

<400> 51
ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc tgg aca   48
Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
  1               5                  10                  15

acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att cct   96
Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
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            20                  25                  30

tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt ctt   144
Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
        35                  40                  45

ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gat gca ctg   192
Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
    50                  55                  60

ttt gtg aag gtc ctg cga tcg cca tagccgttca cagccaaacc actgacattc  246
Phe Val Lys Val Leu Arg Ser Pro
65              70

ctccatgtcc tcacggctgg atttctctct ggaaaggatt ttcattcatc atgaaagcct 306

attccatcaa ctgtgaaagc tggggaatta gaaaaaataa taagtcgctg tcaggtgtgc 366

atgaagaaaa gacactgaag ctaaaaaaga cagcagaact gctatttttc atcctaaaga 426

acaaagtaat gacagaacat gctgttattt aggtattttt ctttaaccaa acaatattgc 486

tccatgatga cttagtacaa a                                           507


<210> 52
<211> 72
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: GPDIII-V

<400> 52
Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
  1               5                  10                  15

Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
            20                  25                  30

Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
        35                  40                  45

Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Asp Ala Leu
    50                  55                  60

Phe Val Lys Val Leu Arg Ser Pro
65                  70


<210> 53
<211> 659
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: HMBP-21

<220>
<221> CDS
<222> (37)..(627)
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<400> 53
gaaaacagtg cagccacctc cgagagcctg gatgtg atg gcg tca cag aag aga 54
Met Ala Ser Gln Lys Arg
1 5

ccc tcc cag agg cac gga tcc aag tac ctg gcc aca gca agt acc atg 102
Pro Ser Gln Arg His Gly Ser Lys Tyr Leu Ala Thr Ala Ser Thr Met
10 15 20

gac cat gcc agg cat ggc ttc ctc cca agg cac aga gac acg ggc atc 150
Asp His Ala Arg His Gly Phe Leu Pro Arg His Arg Asp Thr Gly Ile
25 30 35

ctt gac tcc atc ggg cgc ttc ttt ggc ggt gac agg ggt gcg cca aag 198
Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly Asp Arg Gly Ala Pro Lys
40 45 50

cgg ggc tct ggc aag gta ccc tgg cta aag ccg ggc cgg agc cct ctg 246
Arg Gly Ser Gly Lys Val Pro Trp Leu Lys Pro Gly Arg Ser Pro Leu
55 60 65 70

ccc tct cat gcc cgc agc cag cct ggg ctg tgc aac atg tac aag gac 294
Pro Ser His Ala Arg Ser Gln Pro Gly Leu Cys Asn Met Tyr Lys Asp
75 80 85

tca cac cac ccg gca aga act gct cac tat ggc tcc ctg ccc cag aag 342
Ser His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys
90 95 100

tca cac ggc cgg acc caa gat gaa aac ccc gta gtc cac ttc ttc aag 390
Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys
105 110 115

aac att gtg acg cct cgc aca cca ccc ccg tcg cag gga aag ggg aga 438
Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly Arg
120 125 130

gga ctg tcc ctg agc aga ttt agc tgg ggg gcc gaa ggc cag aga cca 486
Gly Leu Ser Leu Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln Arg Pro
135 140 145 150

gga ttt ggc tac gga ggc aga gcg tcc gac tat aaa tcg gct cac aag 534
Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp Tyr Lys Ser Ala His Lys
155 160 165

gga ttc aag gga gtc gat gcc cag ggc acg ctt tcc aaa att ttt aag 582
Gly Phe Lys Gly Val Asp Ala Gln Gly Thr Leu Ser Lys Ile Phe Lys
170 175 180

ctg gga gga aga gat agt cgc tct gga tca ccc atg gct aga cgc 627
Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser Pro Met Ala Arg Arg
185 190 195

tgaaaaccca cctggttccg gaatcctgtc ct 659

<210> 54
<211> 197
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: HMBP-21

<400> 54
Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His Gly Ser Lys Tyr Leu
1 5 10 15

Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe Leu Pro Arg
20 25 30

His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly
35 40 45

Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys Val Pro Trp Leu Lys
50 55 60

Pro Gly Arg Ser Pro Leu Pro Ser His Ala Arg Ser Gln Pro Gly Leu
65 70 75 80

Cys Asn Met Tyr Lys Asp Ser His His Pro Ala Arg Thr Ala His Tyr
85 90 95

Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro
100 105 110

Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro
115 120 125

Ser Gln Gly Lys Gly Arg Gly Leu Ser Leu Ser Arg Phe Ser Trp Gly
130 135 140

Ala Glu Gly Gln Arg Pro Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp
145 150 155 160

Tyr Lys Ser Ala His Lys Gly Phe Lys Gly Val Asp Ala Gln Gly Thr
165 170 175

Leu Ser Lys Ile Phe Lys Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser
180 185 190

Pro Met Ala Arg Arg
195